

SECTION ONE

GENETICAL STUDIES OF THE PHENOMENON OF MEIOTIC DRIVE
WITH REFERENCE TO THE EFFECT OF TEMPERATURE.

SECTION TWO

CYTOGENETICS OF SUPERNUMERARY CHROMOSOMES IN THE
LOCAL POPULATIONS OF SHORT-HORNED GRASSHOPPER
PHAULACRIDUM VITATUM.

A thesis submitted by

ALAN ABDUL AZIZ

in partial fulfilment of the requirements
for the degree of

BACHELOR OF SCIENCE WITH HONOURS
DEPARTMENT OF BOTANY
UNIVERSITY OF TASMANIA
HOBART

Alan Aziz

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It is my pleasure to thank my supervisors, Professor W.D. Jackson and Mr. D.S.M. Cheung for their help and encouragement during the year. Mr. Cheung also supplied some slides of P. vitattum for the work on chiasma frequencies. Sincere thanks go to Dr. W.J. Peacock of the C.S.I.R.O. in Canberra for Drosophila stocks.

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General Summary

This thesis consists of two principal sections. The first deals with studies on the phenomenon of Meiotic Drive. This includes a reading thesis, which is largely a review of the current literature on the subject. Results of research on the effect of temperature on Meiotic Drive are presented. Two stocks of D. melanogaster showing Segregation Distortion (SD) and Nonrandom Segregation were used.

The second section deals with the cytogenetics of supernumerary (B) chromosomes found in the local population of short-horned grasshopper, Phaulacridium vittatum. The study includes an estimation of the frequency of B chromosome carriers in the female population, distortional segregation of the B with respect to the X chromosomes, and also the influence of B chromosomes on chiasma formation.

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SECTION ONE

PART I

READING THESIS: MEIOTIC DRIVE

I. INTRODUCTION

Segregation of alleles in spermatogenesis and oogenesis, is governed by the Laws of Mendelian Inheritance. Thus, a heterozygote for alleles A and a produces gametes of equal frequencies of each of the alleles. However, there are instances when this 1:1 ratio is not observed among the offspring of a heterozygote although it is evident in the gametic population. This deviation does not challenge the Mendelian Laws of Inheritance, but merely confuses them. Sandler and Novitski (1957) proposed the term meiotic drive for those instances in which the production of the two classes of gametes are not equal despite normal meiosis

"...such a pattern of behaviour will drastically alter frequencies of alleles in a population; where such a force, potentially capable of altering gene frequencies, is a consequence of the mechanics of meiotic divisions, we suggest the name meiotic drive be applied..."

Sandler and Novitski also distinguished meiotic drive from the somewhat similar phenomena of gametic selection and gametic competition. Meiotic drive does not depend on the genotype of the gametes or their fitness.

There are many known cases of meiotic drive in natural populations as well as in laboratory stocks in both plants and animals. The first case to be recorded is perhaps the "sex-ratio" phenomenon

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Sandler and Novitski also distinguished meiotic drive from the somewhat similar phenomena of gametic selection and gametic competition. Meiotic drive does not depend on the genotype of the gametes or their fitness.

There are many known cases of meiotic drive in natural populations as well as in laboratory stocks in both plants and animals. The first case to be recorded is perhaps the "sex-ratio" phenomenon

in D. obscura by Gershenson (1928). He observed that in certain crosses of males carrying a special X chromosome found in wild populations, the number of females recovered among the progeny far exceeded the number of males. This, as Gershenson showed, was not due to zygotic death. The abnormal behaviour of the X chromosome was due to certain factor(s) present on the chromosome. This type of meiotic drive is referred to as genic meiotic drive, since "genes" are involved.

In most instances, the degree of meiotic drive ranges from 60% to 100%. It is generally believed that this variation is dependent to a large extent on the action of modifier genes.

II. MEIOTIC DRIVE IN ANIMALS

Most known cases of meiotic drive are found in animals for no apparent reason. In Drosophila, meiotic drive is mostly confined to the male. The only known case in Drosophila female, is the preferential recovery of the physically shorter member of a heteromorphic (structurally different) pair of chromosomes in the egg nucleus. This phenomenon, known as "nonrandom disjunction", is therefore a chromosomal meiotic drive and no specific genetic factors are known to be involved. Examples are found in females heterozygous for aberrations such as deficiencies (Novitski 1951), translocation (Zimmering 1955) and in females having attached-X chromosomes (Weltman 1954) and other compound chromosomes (Novitski and Sandler 1956).

In Drosophila males, a number of different types of meiotic drive are known viz. nonrandom segregation, segregation-distortion, deviant sex-ratio and recovery disrupter. Recently, sex-ratio condition has been found to exist in wild populations of the mosquito Aedes aegypti. The genetics of these cases in Drosophila and Aedes males will be discussed in considerable detail in the following pages.

a. NONRANDOM SEGREGATION - In (1) sc^4-sc^8

The first case to be considered is Nonrandom Segregation in Drosophila melanogaster males with a deficient In (1) sc^4-sc^8 X chromosome and a normal Y.

This inversion X chromosome is derived from two X chromosomes a) In (1) sc^4 , a long inversion of the X with most of the basal heterochromatin including the locus bb and Muller's block A located proximally and b) In (1) sc^8 , also a long inversion of the X with a large portion of the basal heterochromatin distally located. Their distal breakpoints are similar and their proximal breakpoints in the basal heterochromatin are such that the crossover product is deficient for the 'bobbed' (bb) region and also the Muller's block A heterochromatin portion (Fig. a). This chromosome, although only about 2/3 as long as a normal X chromosome physically, is nearly similar to the latter in genetic length. Included in the deficiency are regions which are normally involved in pairing with the Y chromosome ('collochores' of Cooper, 1959). Thus males with the inversion are expected to produce non-disjunctional gametes, nullo (no X or Y) and XY. This was verified by Sandler and Braver (1954) who found a high percentage of nullo and XY gametes. However, the proportions of complementary classes within disjunctional (X and Y) and non-disjunctional (nullo and XY) gametes were not equal. X and 0 were in excess of Y and XY respectively. Morgan, Bridges and Sturtevant (1925) suggested chromosome loss to be responsible for the unequal

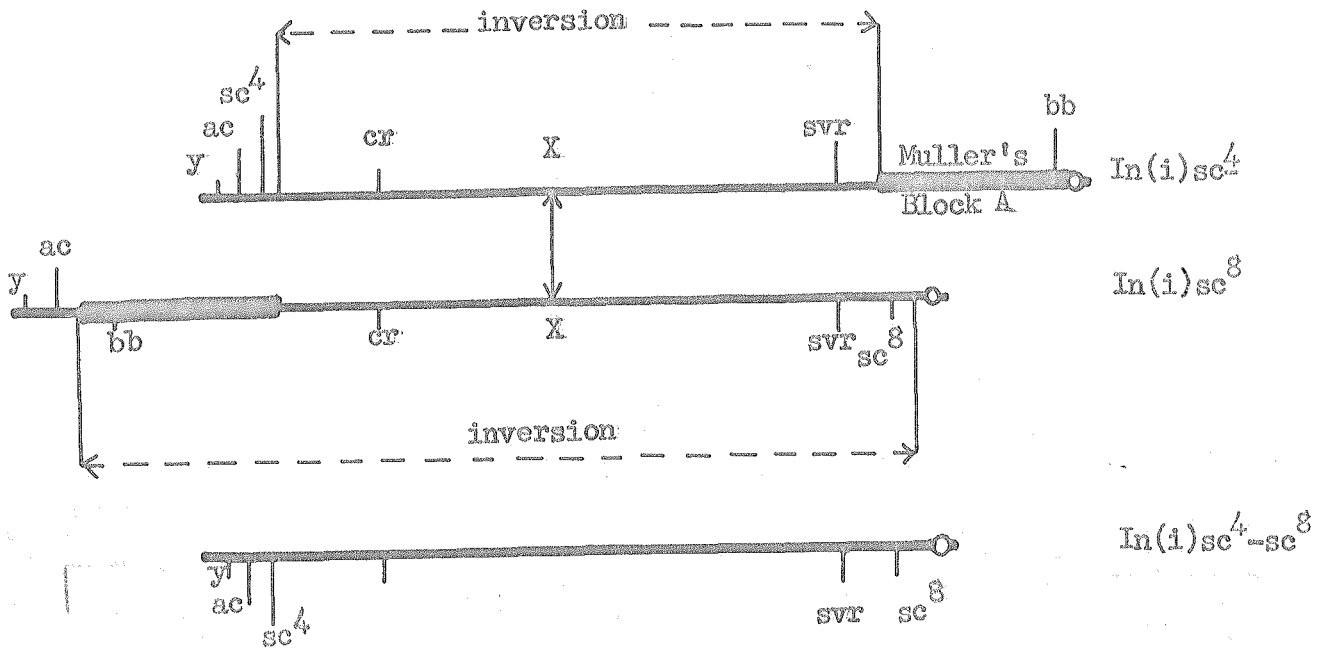


Figure a. Diagram showing the inversions sc^4 and sc^8 and the crossover product inversion sc^4-sc^8 . The centromere is shown as a circle and the crossover region at X. The extent of the inversion in each X chromosome is indicated by broken lines (modified after Swanson, 1957).

frequencies of recovery of exceptional males and exceptional females in D. melanogaster. They believed that "...primary non-disjunction is initiated by some unusual local difference in consequence of which the synapsed X's lag upon the reduction spindle and separate from each other only with difficulty." It was argued by Sandler and Braver that this peculiar result of unequal frequencies in the case of $In(i) sc^4 - sc^8$ was due to meiotic loss at anaphase I of the X and Y chromosomes which were unpaired at metaphase I. This loss amounted to nearly 42% in the Y and about 0.3% (not significantly

different from O) in the X chromosome.

The idea of meiotic loss of the Y chromosome was further investigated by Zimmering (1963) who showed that males carrying In(1) $sc^4 - sc^8$ X/Y which had been raised at a lower temperature at 18°C rather than at 26°C , produced gametes of X and Y, and O and XY in frequencies approaching equality. He suggested that this was due to a decrease in meiotic loss of the Y chromosome at the lower temperature. He also obtained parallel results when he employed a specially constructed Y chromosome (an attached Y-partial X chromosome, $X^{P.L.Y^S}$). This chromosome had the base of the X and both arms of the Y and it was univalent in prophase, since it lacked a homologue. Zimmering inferred from this that (very obviously) the different rate of loss of the Y at the different temperatures is not due to synapsis. He thus suggested that a Y chromosome underwent a high or low rate of meiotic loss and this loss was independent of synapsis.

Novitski and Sandler (1957) made a remarkable deduction from their experimental analysis. They concluded that not all sperms of Drosophila are functional. Although working with males carrying the translocation $T(1,4)B^S$, Novitski and Sandler speculated that this condition was not only limited to the translocation stock, but could be generally true in all Drosophila males, being revealed only when certain experimental conditions were achieved. However, this finding was not taken seriously since some argued that even enenploid

eggs and sperms from translocation heterozygotes were known to function irrespective of their chromosomal contents. Despite this, the idea that not all sperms are functional in Drosophila, has been successfully interpreted and consequently it has effected a more positive understanding of the mechanism involved in the peculiar phenomenon exhibited by the $\text{In}(1) \text{sc}^4 - \text{sc}^8 \text{X/Y}$ males. Peacock (1963) is mainly responsible for this. Studying the cytogenetics of the males, he found that there was no meiotic loss of the Y chromosomes. The $\text{sc}^4 - \text{sc}^8 \text{X}$ was often found to be incapable of synapsis with its Y homologue at M I. The probability of synapsis seemed to be dependent upon the particular Y chromosome present, the background genotype and external factors such as culture temperature. This failure to synapse resulted in the formation of non-disjunctional gametes. From his observations, Peacock inferred that the frequency of synapsis at M I equalled the frequency of A I disjunction and that the frequency of failure of synapse equalled that of non-disjunction.

When the X and Y failed to synapse, their anaphase movement was highly non-random; usually both moved toward the same pole. The frequencies of complementary classes in both disjunctional and non-disjunctional gametes were equal at the end of meiosis, typifying a normal meiotic process. Each sperm bundle was found to contain 64 normal sperms and no significant zygotic death was observed. Genetic determinations of the frequencies of non-disjunctional gametes were in agreement with those from cytological determinations.

Non-disjunctional gametes were found to vary over a wide range from 40% to 6%. Despite all these findings which showed normal meiotic and post-meiotic processes taking place, there was still a significant difference in the frequencies of complementary classes. These frequencies were relatively constant in the various crosses in the same physical environment and appeared to be independent of the amount of non-disjunction.

In the light of these observations, Peacock proposed that there is preferential segregation of unpaired X and Y chromosomes, both are usually included in the non-functional poles of Novitski and Sandler. For an XY gamete to function, both X and Y chromosomes must be included in a functional pole. This explains the great excess of nullo gametes over XY gametes. Preferential segregation into functional gametes also accounts for the ratio of X to Y in disjunctional gametes. The X chromosome has a greater probability of being orientated toward the functional pole than has the Y when both chromosomes are associated in a bivalent. This explanation is also valid in accounting for the results obtained by Zimmering (1963). At the lower temperature, therefore, the movements of paired and unpaired chromosomes were random.

Although it is known that the X is preferentially segregated toward the functional pole and that the X and the Y when unpaired are preferentially segregated toward the non-functional pole, the actual mechanism of these processes including factors governing the

movement of these chromosomes at anaphase is still unknown.

Nevertheless, Peacock has shown that meiosis involving the

In(1) $sc^4 - sc^8$ X/Y is strictly normal and this is sufficient evidence for the fact that this non-random segregation of X and Y chromosome is an example of meiotic drive.

b. SEGREGATION-DISTORTION - SD

The phenomenon of Segregation-distortion in Drosophila first discovered in wild populations in 1956, is another case of meiotic drive. It is due to a locus referred to as Segregation-distorter (designated S.D.), located near the centromere in the right arm of chromosome II. The genetics and nature of S.D. have been studied in the laboratory by Sandler et al (1959) and Sandler & Hiraizumi (1959, 1960a).

The S.D. bearing chromosome is recovered more often than its homologue which carries the SD^+ . In many stocks, the value of k i.e. the proportion of SD bearing flies in total recovered offsprings, is quite constant and has a value of 0.95. The phenomenon, however, takes place only in heterozygous males (SD/SD^+); homozygous SD males and all SD females whether homozygous or heterozygous do not exhibit the phenomenon. It appears that synapsis between the SD locus with its homologue SD^+ is an essential prerequisite for segregation-distortion. This is believed to be true since structural abnormality in the SD^+ bearing chromosome in the SD region reduces segregation-distortion (in some cases, is usually eliminated altogether). Thus, when SD is heterozygous with a structurally rearranged homologue, such as the Curly inversion, In (2LR)Cy, distortion is greatly suppressed.

The fact that no distortion or sterility is observed when SD bearing chromosomes are made homozygous in males, suggests that an

SD bearing chromosome is insensitive to the action of another SD element. Possibly, the SD elements act on each other with the same degree such that the net effect is zero, and thus no segregation-distortion.

All the SD bearing chromosomes collected from natural populations, contain an inversion in the right arm of chromosome II which causes a reduction in crossing over in that arm (crossing over in the left arm, IIL, is normal). However, occasional recombinations occur between *cn* (cinnabar) and *bw* (brown) loci in the IIR (right arm of chromosome II). Such recombinants that contain the SD locus have varying *k* values among test males. When the variation in *k* values is very large, the recombinants are classified as "unstable", and when it is small, the recombinants are classified as "semi-stable". The original SD line is termed "stable" line. The difference in stability between any two lines is, however, not distinct. Experiments involving selection of offsprings of stable, unstable and semi-stable parents for *k* values, revealed that neither semistable nor unstable SD line can be made stable merely by selection. However, conversion of semistable to unstable is possible (although the reverse is not) when the semistable SD line is passed through both males and females. The fact that the conversion is possible through males suggests that it does not require crossing over. Consequently, the semistable SD line does not differ from the unstable SD element by a modifier. Since neither semistable nor unstable can be made stable or vice-versa, it is believed that the

stable SD element is different from its unstable and semistable counterparts by merely a single modifier. The presence of this modifier renders the SD line stable and in its absence, the SD is either semistable or unstable.

Sandler and Hiraizumi (1960a) proposed that (through evidence from recombination experiments involving *cn* and *bw* loci) this stabilising modifier, designated St(SD), must be located at the tip of the right arm of the second chromosome (II_R). St(SD) is found to be capable of completely stabilising semistable SD lines but not unstable lines, although in the latter case, it increases the distorting action. Losing St(SD) by recombinants, convert them first into semistable and then into the unstable state spontaneously.

The structure of SD region was studied by Sandler and Hiraizumi (1960b). They proposed that the SD locus lies between *cn* and *pr* (purple) loci with the SD locus very close to the centromere. SD is very closely linked to a locus termed Activator of SD designated Ac(SD) which is located to its right. Hiraizumi and Nakazima (1967) found that this Ac(SD) is 0.5-1.0 unit away from the SD locus (Fig. b). Both these elements thus lie in a very small chromosomal segment (aberration). This chromosomal aberration causes a suppression in crossing over in the SD region. When crossing over does take place, the rare recombinant SD Ac(SD)⁺ produces a weak distorting effect with *k* averaging a value of about 0.8. The other recombinants SD⁺Ac(SD) shows no distorting effect at all. These recombinants are therefore partially and wholly

insensitive to the action of SD. Based on these rare recombinants and their non-distorting nature, it is concluded that the $Ac(SD)$ is necessary for SD to operate and that they must be present in the coupling state with SD.

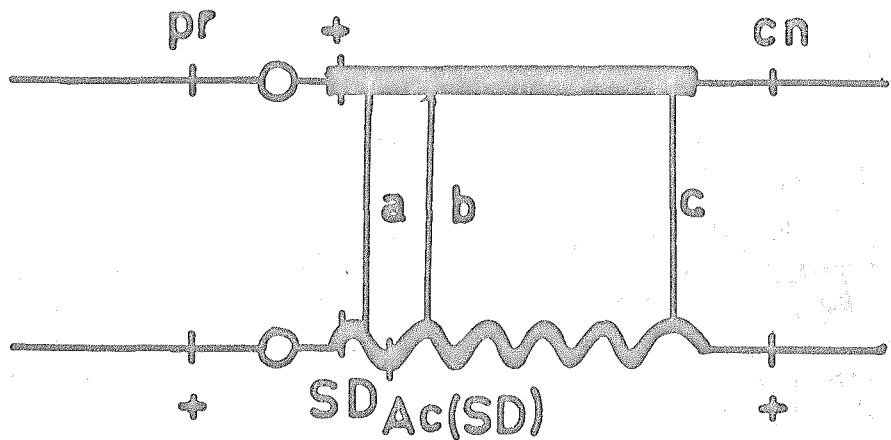


Figure b. A diagram showing the SD region. The heavy lines show homologous regions within SD region; the wavy line is the aberrant segment responsible for the reduction in crossing over and for insensitivity of the original SD-bearing chromosome, and a, b, and c are crossover regions between pr and cn (after Sandler and Hiraizumi 1960b).

Conditional-distortion

As mentioned earlier, Drosophila females do not play any part in segregation-distortion. However, an interesting phenomenon is associated with them with respect to SD. It is found that in certain lines, if the SD element is inherited from a female parent, then in a fraction of F1 male sibships, only one half of the heterozygous sons produce segregation-distortion. The other half produce normal segregation. In the F2, irrespective of whether or

not their father showed segregation-distortion, all males show abnormal ratio. Sandler and Hiraizumi (1959) termed this phenomenon "Conditional distortion". And females which produce heterozygous sons of which only half exhibit segregation-distortion, are referred to as being "conditioned". Sandler and Hiraizumi (1961a) made further studies on this phenomenon and also proposed an explanation to its cause. It is thought that in males carrying SD element, the element causes the X chromosome to be induced into a specific modifier. This specific induction seems to be stable and comes about without physical contact between SD element and the induced modifier of the X. The induced X chromosome in turn suppresses the action of SD. Hence a conditioned female (which is produced by a normal female and SD male receives one induced X chromosome from her father; her other X is normal. Half her sons therefore receive an induced X and thus these males produce normal segregation ratios. When both types of sons are crossed to normal females, all male offsprings produced will receive a normal X from the normal mother and hence all will show segregation-distortion. However, the exact nature of the proposed conditioned state is unknown.

Cytogenetic model of S.D.

The cytogenetic basis of segregation distortion was investigated by Sandler et al (1959) who worked on the assumption that the SD^+ had a lethal effect and as such would be eliminated during meiosis by a special mechanism. They postulated that during synapsis of SD

and SD^+ -bearing chromosomes at prophase I, the SD element induces a misreplication (breakage) of the SD^+ bearing chromosome in a region opposite the SD. This break becomes a sister-strand fusion which at anaphase II produces a chromatid bridge. The chromatid bridge and its subsequent fragments following breakage will be duly eliminated (Fig. c).

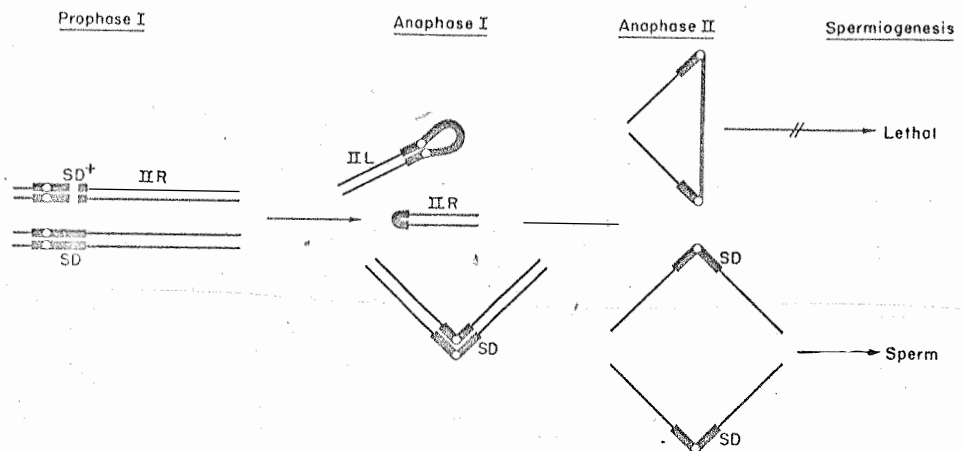


Figure c. A diagrammatic model to account for segregation-distortion, showing the elimination of the SD^+ bearing chromosome (Sandler et al 1959).

This hypothesis was supported by some preliminary cytological observations which were by no means conclusive. Sandler et al (1959) reported that bridges and dicentrics were consistently observed at metaphase II but no acentrics were reported seen which was quite unusual. Nevertheless, data supporting the breakage model were available from studies by Crow et al (1962) and Hiraizumi (1962).

The breakdown model hypothesis was short-lived, due to work by Peacock and Erickson (1965) who found that cytological examination

revealed no evidence for the validity of the hypothesis. Peacock and Erickson failed to observe the dicentric loops which Sandler and colleague consistently found. Instead, the SD system mechanism paralleled that of the nonrandom segregation of $sc^4 - sc^8$ in that meiosis was normal. Studies on sperm bundles, maturation, transfer and storage did not show any difference between SD and SD^+ . Peacock and Erickson thus proposed that segregation-distortion is due to preferential segregation of the SD-bearing chromosome into the functional pole and thus leaving the homologous SD^+ bearing chromosome to be included in the nonfunctional pole because the SD bearing chromosome always separates reductionally from SD^+ bearing chromosome at first division and since nearly all functional gametes contain SD bearing chromosome ($k \approx 0.996$ in most instances), it is reasonable to conclude therefore that one pole at first meiotic division produces two functional sperms while the other produces two non-functional sperms. It follows that there must be some special mechanism involved, probably an orientation of the SD/SD^+ bivalent at anaphase I such that the SD complement, in this orientation, has a greater probability of going toward the functional pole than has the SD^+ complement. However, nothing more is known about this orientation.

Sex-ratio in association with SD

Novitski and Ehrlich (1966) reported that when the non-SD chromosomes are involved in a Y translocation, the action of SD may be drastically modified or even reversed. They also reported that

severe suppression of SD action might also take place if the SD heterozygous males carry attached \overline{XY} X chromosome. This is in fact a modification of SD action by a non homologue. Sandler and Hiraizumi (1961) also realised similar observations when they studied conditional segregation in Drosophila. Hiraizumi and Nakazima (1967) also observed the association of SD with non-homologues. They found that SD heterozygous males showed slight but consistent sex-ratio deviation. When SD/SD^+ males were mated to the standard test cnbw females, in the F_1 , more females were recovered among the small cnbw progeny, while more males were recovered among the large SD progeny. However, SD/SD^+ females did not show similar results.

Hiraizumi and Nakazima explained this non-random assortment between the second and the sex-chromosomes in the light of the meiotic mechanisms of the SD and sex-ratio as proposed by Peacock and Erickson (1965) and Novitski et al (1965). The SD locus is believed to have some sort of homology with some parts of the X in such a way that between them there is competition to reach the functional pole. When SD goes to the functional pole, the probability of the X reaching the same pole is reduced. Similarly, when the X chromosome reaches the functional pole, the probability of the SD reaching the same pole is in turn reduced. Both $Ac(SD)$ and $St(SD)$ seem to "inactivate" the X chromosomes by reducing the latter's probability of reaching the functional pole.

c. DEVIAANT SEX-RATIO IN DROSOPHILA

Sex-ratio condition i.e. differential recovery of sex, has been studied in a number of species of Drosophila as in D. obscura by Gershenson (1928). Sturtevant and Dobzhansky (1936) found a wide distribution of sex-ratio in wild populations of D. obscura group which include D. affinis, D. athabasca, and D. azteca. In all these three species, the offsprings of sex-ratio males consist of mainly daughters and few or no sons, regardless of the genetic constitution of the female parents. Novitski (1947) working with D. affinis also found the same phenomenon. In addition, he also found in the same species a recessive gene which reversed the effect, giving an excess of male progeny or male sex-ratio. Female sex-ratio has also been found in D. paramelanica (Stalker 1960) and D. simulans (Faulhaber 1967).

In females, sex-ratio is due to males heterozygous for an X chromosome carrying the sex-ratio "gene" which produce an excess of X-bearing sperms and very few Y-bearing ones. As a result, more females are recovered in the progeny. Sturtevant and Dobzhansky (1936) made cytological studies of sex-ratio in Drosophila pseudoobscura in an attempt to account for the abnormal mechanism involved. They suggested that at the first meiotic division the X chromosome in sex-ratio male undergoes an extra replication thus forming four X chromatids. These are then distributed among the four spermatids and hence all sperms are X-bearing. The Y chromosome degenerates at the first division and is eliminated in subsequent

meiotic stages.

Stalker (1961) who studied sex-ratio in D. paramelanica found that in the natural habitats in which the species occurred, two forms were identified, the Northern-type and the Southern-type. The Northern-type sex-ratio could be suppressed by Southern-type Y chromosomes but not by its own type Y while the Southern type was not suppressible by either type Y chromosome. Both types of sex-ratio were, however, suppressible by a less effective suppressor system. This system of suppressors were believed to be autosomal. The sex-ratio condition is due to a "gene" designated *sr* which is located on the X chromosome. Further studies by Stalker showed that this *sr* gene is related to a system of inversions in a fairly complex manner, similar to the situation reported by Sturtevant and Novitski (1941) in D. melanica and by Darlington and Dobzhansky (1942) in D. pseudoobscura. In D. paramelanica, the sex-ratio X chromosome differs from the "Standard" by having at least two inversions (A and B) in the left arm and two or three (C and BA complex) in the right (Fig. d). The two inversions A and B in the left arm show rare

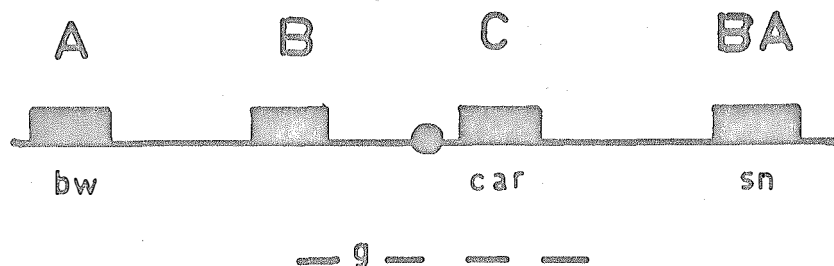


Figure d. Sex-ratio X chromosome of D. paramelanica showing multiple inversions (Stalker 1961).

crossing over with each other and with the inversion C of the base of the right arm. Crossing over may also occur between inversions C and the BA complex. Inversions A and B are very close together in the BA complex and crossing over between them has not been observed.

Recombination experiments involving these inversions show that sex-ratio X chromosomes of both Northern and Southern-types carrying inversions BA complex or C as well as BA complex in the right arm, do retain the sex-ratio condition. This suggests that the *sr* gene is in fact located in the right arm of the X in this region of BA complex. The suppressibility of Northern-type sex-ratio X by a Southern-type Y is found to be associated with inversions A and B in the left arm. Suppression of sex-ratio in the Southern-type can be achieved by replacements of the left arm of Southern-type sex-ratio X by "standard" left arm. The Southern-type can thus be suppressed by its own type Y; in other words it is transferred into a Northern-type. This therefore suggests that the difference between the two types lies on the genetic characteristics of the left (autosomally derived) arm of the X. However, any similar replacement in the left arm of a Northern-type will suppress the sex-ratio trait completely. Thus, any breakdown in the inversion system would deprive the X chromosome of meiotic drive advantage. This is why, in natural wild populations of the species, such signs of breakdown of the system which is reflected by the presence of recombinants are very rare.

In D. simulans, the condition of sex-ratio is due to a recessive factor (sxr) located between hairless (H) and petit (pe) on chromosome II. Time-temperature sensitivity experiments show that the primary action of this autosomal recessive factor starts prior to meiosis, the product of which interacts with a Y chromosome factor during spermiogenesis. As in D. pseudoobscura, primary non-disjunction also occurs in D. simulans. The XO males produced by non-disjunction do not show the morphological abnormalities of spermatids which are characteristic of the XY sex-ratio males (some spermatids in XY sex-ratio males have undeveloped heads). This suggests that the abnormal sperm head development apparently requires the presence of the Y chromosome (Faulhaber 1967).

Cytogenetic Basis of sex-ratio

The model proposed by Sturtevant and Dobzhansky to account for sex-ratio in Drosophila has been found to be incorrect. The X chromosome has not been observed to undergo an extra replication at first prophase, although such a unique situation does in fact occur in the coccid Llaveilla (Huges-Schrader 1940). Instead, Novitski et al (1965) found that the X chromosome in D. pseudoobscura is preferentially segregated into the functional pole at anaphase I. The Y chromosome is usually included in the non-functional pole ensuring that all progeny are females. Parallel results are also observed in cytological analysis of a related species, D. athabasca.

d. RECOVERY-DISRUPTER - RD

From population cages of D. melanogaster (Wallace 1956), which had been subjected to low intensity irradiation for about 200 generations, two lines were obtained in which the chromosomes derived from the irradiated population were recovered in more than half of the offsprings, Novitski and Hanks (1961). One of them contained an irradiated chromosome II, which was found in the F_1 of heterozygous male parents with frequencies ranging from 53% to 80%. This deviation persisted for 6 consecutive generations. But the line is neglected and consequently lost. The other line involved an irradiated X chromosome. When present in a parental male, it appeared in 56% to 64% of his offsprings. This line was inbred for about 20 generations, at the end of which the mean recovery of the X chromosome was found to be a constant, approximating 67%. This deviation in sex-ratio was not simply due to differential mortality of the male progeny caused by the irradiated X chromosome. This was shown by crossing such males (those producing differential sex-ratio) to attached-X females. In such a cross, the transmission of the irradiated X of the father was effected through his sons only. The progeny of this cross were found to contain significantly more males than females, reflecting that the irradiated X chromosome was recovered in excess of the Y, and more significantly, that it was in the presence of the irradiated X chromosome that the deviation in sex-ratio was observed. This new inbred line and its three components, autosomes, X and Y were referred to as Recovery Disrupter,

designated RD. X^a and Y^a were symbols for RD X and RD Y chromosomes respectively, and A^a represented most of the RD autosomes, Hanks (1964). Previously, the line was named "29 G" by Hanks (1961) and Erickson and Hanks (1961). The phenomenon, due to some pre-zygotic mechanism, manifested itself only in males.

Hanks (1964) studied the nature of this Recovery Disrupter and made the following findings. Each of the three components (X^a , Y^a and A^a) has a definite influence of recovery of sex chromosomes. Y chromosomes are somewhat different in their recovery of sex chromosomes effect. Since A^a influences sex chromosome recovery rate, there must be certain mechanism present which cause this interchromosomal effect. Hanks believed that the 67% recovery of XY is not the effect of any single RD factor (i.e. X^a or Y^a or A^a) but is in fact due to the interaction of all three components whose combined effect is generally multiplicative. The active site on the X^a chromosome is believed to be located between car (carnation) and su-f (suppressor of forked) near the euchromatic-heterochromatic junction. Recovery-disrupter effect is enhanced by modifiers which are present on the other chromosomes of the complement.

Cytogenetic model for Recovery-disrupter

The mechanism of RD action was studied by Erickson (1964). Unlike the three previous cases of meiotic drive i.e. nonrandom segregation, segregation-distortion and sex-ratio, the phenomenon of Recovery-disrupter does not involve preferential segregation of chromosomes. Cytological observations revealed that it involves a

depression in the recovery of the Y chromosome, somewhat similar to the original model proposed by Sandler et al (1959) to account for SD. Erickson proposed that there is an action against the Y chromosome during an early stage, even before anaphase I. Time-temperature sensitivity experiments by Erickson and Hanks (1961) show that the definitive action occurs at prophase I. Occasionally, the Y chromosome breaks, usually in the long arm and this Y fragment remains on the spindle at anaphase I. The exact mechanism involved is not fully understood. It is speculated that irradiation causes a rearrangement in the X heterochromatin. At prophase I, this X synapses with the Y in a manner such that the rearranged X heterochromatin is involved in pairing with the long arm of the Y. A small deletion in the X heterochromatin would effect a "hump" in the Y; a small insertional translocation or a duplication of a segment would cause stretching of the Y in the corresponding region; and an inversion of the X would produce a loop in the Y which would cause some stress in the chromatids. All these three cases would cause a stretching or a loosening of coils. This would occasionally break at anaphase I but more frequently, the break takes place at anaphase II. During the second meiotic division, these Y fragments protrude from the nucleus or get isolated in the cytoplasmic contents of the spermatids. These fragments lead to lethal action during spermiogenesis. Observations of sperm bundles showed that approximately 48 sperms were countable as compared to 64 in normal control sperm bundles.

RD activity.

Experiments using $X^aY^1Y^2$ males, showed that the normal sex-ratio associated with RD was obtained when segregation of X^a with Y^1Y^2 took place. Here, $R = 50\%$ (where $R =$ the recovery value, defined as the percentage of females). When segregation between X^aY^1 with Y^2 or Y^aY^2 with Y^1 occurred, a deviant sex-ratio was also observed. But in this latter case, the sex-ratio was reversed i.e. more males were found in the progeny ($R = 50\%$). These observations could be accounted for by assuming that in the latter case, some of the X^aY^1 and X^aY^2 gametes were lethal. This could be, most likely, due to the "action" against the Y^1 and Y^2 in the respective gametes by the X^a , rendering lethality to these gametes. The solitary Y bearing chromosome was not affected.

e. DEVIANT SEX-RATIO IN AEDES

In the mosquito Aedes aegypti, sex is determined by a single gene or a small chromosomal segment, designated m . Females are homogametic, mm and males are heterogametic, Mm . Normal segregation in males would therefore produce equal numbers of M and m gametes. Christopher (1960) reported that males were found to be in excess of females, with frequencies ranging from 55% to 65%. This high male ratio is due to a hereditary factor transmitted by males (Craig, Hickey and Van de Hey 1960). Males which produce high male ratios, do so regardless of the genotype of the females they are crossed to. Gametic selection is ruled out and the phenomenon is referred to as male-producing or MP. This phenomenon is another example of meiotic drive. Hickey and Craig (1966) did much to throw light on the nature and genetic basis of this male-producing phenomenon. The male-producing factor is designated Distorter (D) and its absence as d . This factor acts directly at the locus for sex-determination, m ; thus the symbol D or d is written as superscript of M or m .

MP phenomenon is found to be exhibited only by males which are Mm^d . The origin of either chromosome M^D or m^d , (whether transmitted by male parent or female parent) is immaterial. Thus MP can be lost and recovered in sequential generations. The reciprocal genotype $M^d D$ and the other two possibilities $M^D D$ and $M^d d$, all give the normal 1:1 ratio. MP is not observable in females.

Distorter (D) seems to have no distortion effect on autosomes of the complement, hence chromosomes 2 and 3 in $M^D m^d$ males segregate normally. However, all loci on sex-linkage group 1 show segregation typical of MP. Hickey and Craig were able to select MP males which produced distortion as high as 85% to 90%. Because of the deviant segregation of loci on the sex-linkage group, it is believed that the distorter is probably located within the sex-linkage group itself, somewhere to the right of red eye (re). However, its precise location is yet to be determined.

Based on the varying levels of male ratios observed, there are two kinds of MP males (1) those that produce 0 to 9% females and (2) those that produce 20 to 37% females. This difference in distorter action has not yet been accounted for in terms of modifier systems sensitivity. Unlike the SD system, increase in age of an MP male does not alter sex-ratios among its progeny. Varying temperatures have a similar effect on MP condition.

The mechanism of action of distorter is still unknown. However, it has been shown that relatively few female-bearing gametes from MP males succeed in fertilising eggs, and that MP males deplete their sperm earlier than do normal males. This could either be due to meiotic loss of the m-bearing chromosome or to the m-bearing element being preferentially segregated toward the non-functional pole of meiotic anaphase I (if there is such a thing as a non-functional pole in Aedes aegypti males).

III. MEIOTIC DRIVE IN PLANTS

Unlike in animals, only a few cases of meiotic drive in plants are known to this date. Perhaps the most significant of these is the case of preferential segregation of certain chromosomes in Zea mays, since it is a genuine case of drive and that the mechanism of cytogenetic deviation involved is well understood.

An abnormal chromosome 10 was first discovered by Longley (1938) who was studying the karyotype of a number of maize strains of the North-American Indians. This chromosome designated (K10) differs from the normal chromosome homologue (k10) by a variation in chromosome pattern by the presence of a large knob and euchromatin on one end of its long arm. These extra regions have not been found to have homologous regions elsewhere in the maize genome. When either of these chromosomes is studied in a homozygous state, segregation is normal with respect to Mendelian ratios for any loci on this chromosome. However, Rhoades (1942) reported that plants heterozygous for the two chromosomes (K10/k10) produced approximately 70% of the megaspores carrying the abnormal K10 chromosome. This deviation from the 1:1 ratio was not due to any particular gene but found to be influenced by the knobbed region. Such deviant segregation was also found to occur for both chromosomes 6 and 9 in K10/k10 plants, if the homologues were different in their knob constitution (Longley 1945). Hence in chromosome 9, which has a knob in the short arm, and also the genes C, Sh and Wx with C nearest the distal

knob and Wx nearest the centromere, the gene C gives maximum degree of segregation. Wx gives the least segregation while Sh gives segregation value mid-way between C and Wx. These results are in agreement with the preferential segregation of the knobbed chromosome, that genes closest to the knobbed region undergo the greatest degree of segregation.

The chromosome K10, in addition to showing abnormal segregation, also shows other cytogenetic effects. It is found that it effects an increase in crossing over and hence recombination (Rhoades and Dampsey 1957, Kikudome 1959). Rhoades and Dampsey (1966) reported that the increase in recombination was probably due to the chromosome K10 inducing very intimate synapsis. Rhoades and Dampsey also reported that preferential segregation was not confined only to chromosomes 10, 6 and 9, but also occurred in all the chromosomes in the complement if they were heteromorphic pairs and the chromosome K10 was also present.

The size of the knob seems to have an influence on abnormal segregation. Kikudome (1959) found that the recovery of the allele Wd ranges from 68.8% in K10/k10. K^L_9/k_9 plants to 59.1% in K10/k10 K^S_9/k_9 plants. The K^L_9 is a much larger terminal knob relative to the smaller K^S_9 . These results are true when equal recombination takes place between Wd and Wx loci and equal numbers of heteromorphic dyads are formed. This, therefore, shows that the greater the knob is, the greater is the degree of preferential segregation.

Perhaps the most interesting effect the chromosome K10 has is that it induces the formation of "neocentromeres" during the anaphase of the first meiotic division (Rhoades and Vilkomerson 1942) Rhoades 1952). This effect of the chromosome K10 forms the basis of the mechanism of preferential segregation. Rhoades 1952 investigated the role of the neocentromeres in maize homozygous for K10 during meiosis. He found that at MI, when bivalents are co-orientated on the spindle, certain chromosomes extend chromosomal fibres from their distal ends. These neocentric regions (normally such chromosomal fibres are produced only at the true centromere) move poleward in advance to the true centromere. This direct relationship between the precocious activities at AI and the heterochromatin knobs are also observed in the second meiotic division.

In the light of these observations, Rhoades (1952) proposed a cytogenetic model in an attempt to account for the preferential segregation in K10 in maize. Briefly, the two spindles of the second meiotic division of the megaspore mother cell in maize are arranged in tandem. The basal megaspore develops into the female gametophyte while the remaining three degenerate and are thus lost. In maize heterozygous for knobbed and knobless homologues, one arm of some of the disjoining dyads at AI possesses precociously acting chromosomal fibres which are absent in the homologous arm. Neocentromeres are formed from the knobbed arms while none are formed from the knobless arms. These neocentric regions move toward the pole in advance to the true centric regions. In a dyad consisting of one knobbed and

one knobless chromatid, the knobless chromatid faces the equatorial plate while the knobbed chromatin lies closer to the pole. If this orientation persists until the second metaphase such that the knobbed chromatids face the two terminal (outside) poles and the knobless chromatids face the inner poles, then only knobbed chromatins are recovered in the terminal poles, one of which is the functional basal megaspore. In the case of heterozygote $K10/k10$, preferential segregation can only occur when a crossover takes place between the knob and the true centric region. If no crossover takes place, then segregation is essentially 1:1. Crossing over between the knob and the true centromere to produce heteromorphic dyads is an essential qualification for preferential segregation. Preferential segregation is not influenced by the number of exchanges; single and double crossovers give equal degree of preferential segregation. If this hypothesis is valid, then the determining factor of preferential segregation is the orientation of the disjoining dyad at AI. The presence or absence of neocentric formation at MII has no effect since orientation has already been established at AI. This point is confirmed by studies on deficient dyads where random segregation at MII is indicated for heteromorphic dyads in which the deficient knobbed chromatid comes from a di-centric bridge. Employing $K10/k10$ heterozygous for several paracentric inversions, Rhoades (1952) found that at AI, a chromatid which is knobbed and acentric formed as a result of a single c.o. shows neocentric activity. However, the same chromatid fails to show any neocentric

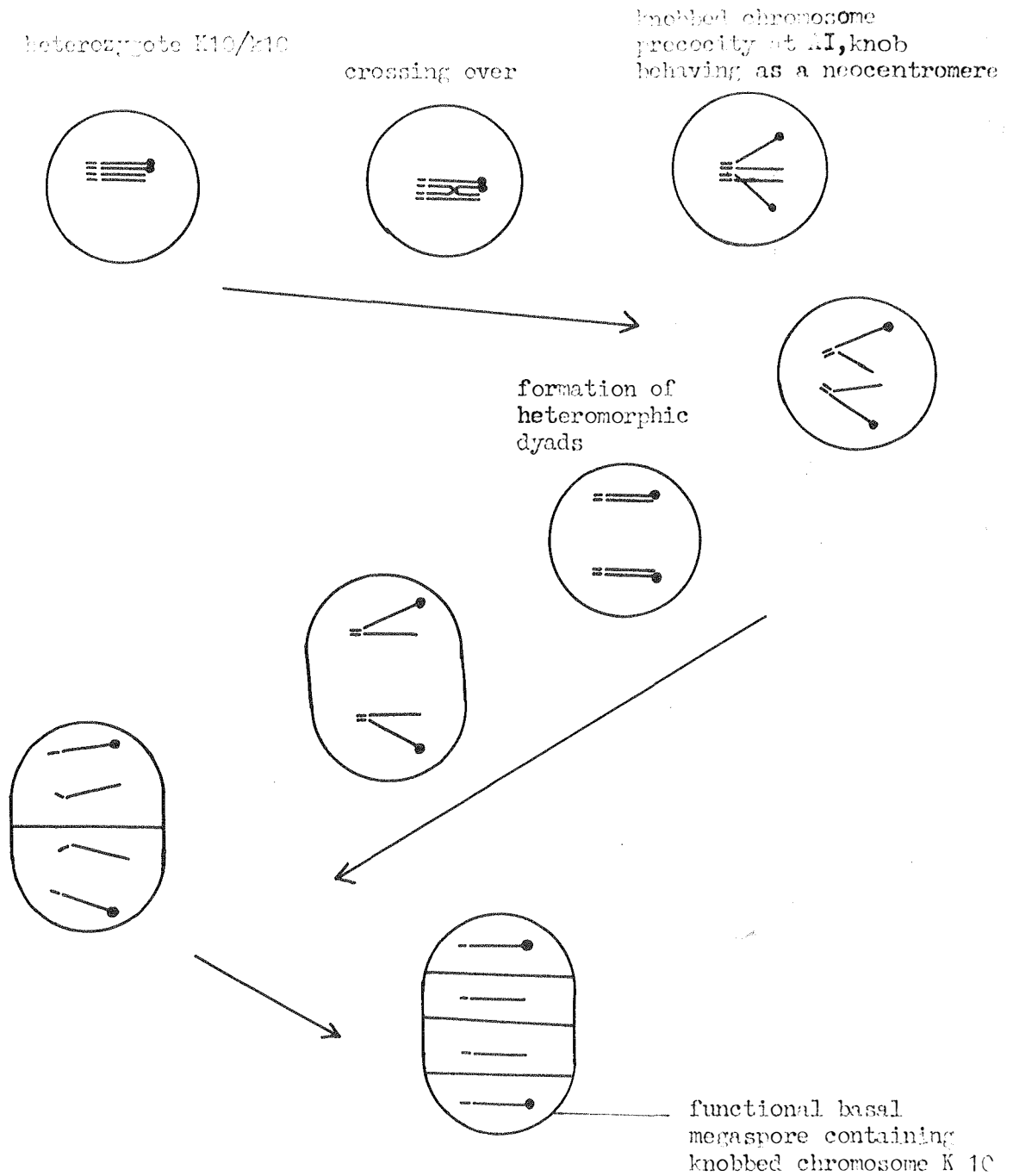


Figure e. Diagram showing preferential segregation of a knobbed chromosome K10 into the functional basal megaspore in *Zea mays*.

activity at AII and thus lies on the equator. Rhoades postulated that the true centromere produces a substance (S) which is essential for the formation of chromosomal fibres and in K10 plants, this substance moves along the chromatid arm towards the neocentromere. This substance is insufficient in quantity at AII, since it is being used up at AI.

IV. POPULATION DYNAMICS AND EVOLUTIONARY IMPLICATIONS

Gershenson (1928) who first realised the phenomenon of sex-ratio speculated that since more X-bearing sperms than Y-bearing ones were functioning in the fertilisation of eggs, the sex-ratio X chromosome would increase in the population and in doing so, would replace the normal X completely. Should this happen, the species would be in danger of extinction because of the scarcity of males. This speculation is of course logical but it does not eventuate in wild populations due to the complexity of genetic systems in action.

As expected, a locus, a chromosomal segment or a whole chromosome which exhibits meiotic drive, has an advantage by virtue of its disproportionate presence in gametes contributing to each generation, and as such can increase in frequency in a population. This is true even if the driven element is associated with genes which impart an effect of reducing the fitness of the organism carrying the driven element. The force of meiotic drive then, is opposed by the force of selection. The effective direction the driven element takes with respect to frequency, will depend on the magnitude of the two forces involved. In man, certain diseases are known to be due to dominant genes, such as thalassemia and sickle cell anemia (Dunn 1953). The frequencies of these conditions were found to be very high in man and could have arisen as a result of an abnormal segregation. This abnormal segregation in man has not been fully understood concerning

its cytogenic properties. Neel (1956) suggested that the high frequency of thalassemia and sickle cell anemia in man could be due to high mutation rates and not by abnormal segregation. Another possibility could be that it is a case of heterozygote advantage (or overdominance). In mice, the frequency of the "t-alleles" for tailless (which is lethal in the homozygous state) is maintained in wild populations at a high level by abnormal segregation. In both cases, man and mice, the abnormal segregation is met with by a reduction in fitness.

It will be recalled in the discussion on sex-ratio (page 19) that D. paramelanica are found in wild populations in two distinct forms viz., Southern-type sex-ratio and Northern-type sex-ratio, and that Southern-type Y chromosome is able to completely suppress the Northern-type sex-ratio gene. Hence if a Southern-type Y chromosome is found among Northern-type populations, through migration of Southern-type sex-ratio flies into the Northern-type populations and through interbreeding between the two, the Southern-type Y will suppress the Northern-type sex-ratio gene. Consequently, the frequency of sex-ratio X will decrease and simultaneously, the frequency of Southern-type Y will increase. The Northern-type Y is constantly lost due to meiotic drive against it and it does not have any suppressing activity of the sex-ratio gene as its southern counterpart has. Because of this, the Southern-type Y chromosome will be found replacing the Northern-type Y, in the Northern-type populations. Stalker (1961) did in fact detect a certain frequency of Southern-type Y chromosome among Northern-type populations.

However, the frequency was low and did not seem to increase i.e. no replacement of Northern-type by Southern-type Y was observed. This suggests that the two types of Y chromosomes differ in some way other than their suppressing ability. It is this other difference (probably a reduction in fitness associated with the Southern-type Y chromosome) that renders the Northern-type Y sufficient adaptive advantage to repel the negative selective force.

Hiraizumi et al (1960) investigated the evolutionary effects of meiotic drive of segregation-distortion in population cages of D. melanogaster. A mathematical model was presented based on models as proposed by Prout (1953) and Sandler and Novitski (1957). A complete mathematical treatment of population dynamics of meiotic drive is complicated since the phenomenon is effective only in one sex. The following mathematical treatment assumes meiotic drive being effective in both sexes to the same degree.

Consider two alleles, A_0 and A , which are the driven element and its normal allele respectively. W_{00} , W_{01} and 1 are the fitness of $A_0 A_0$, $A_0 A$ and AA , and q is the frequency of A_0 .

genotype	proportion f	fitness W	fW
$A_0 A_0$	q^2	W_{00}	$q^2 W_{00}$
$A_0 A$	$2q(1-q)$	W_{01}	$2q(1-q)W_{01}$
AA	$(1-q)^2$	1	$(1-q)^2$

The average fitness of the population is

$$\begin{aligned}\bar{W} &= \sum (fW) \\ &= q^2 W_{00} + 2q(1-q)W_{01} + (1-q)^2 W_{11}\end{aligned}\quad \text{I}$$

If k = the segregation ratio of the heterozygote, then it can be shown that the change in the frequency of q i.e. Δq , is

$$\Delta q = q(1-q) \left[q(W_{00} - 2W_{01} + 1) + (2W_{01}k - 1) \right] \quad \text{II}$$

$$= - \frac{(2W_{01} - W_{00} - 1) q (1-q) (q - \hat{q})}{\bar{W}} \quad \text{III}$$

$$\text{where } \hat{q} = \frac{2W_{01}k - 1}{2W_{01} - W_{00} - 1} \quad \text{IV}$$

When the driven element A_0 , first appears in the population, its frequency q is very small and, to a good approximation, equals to zero. From eq II, $\Delta q > 0$ is only true when $2W_{01}k - 1 > 0$. Thus, the chromosome, or chromosomal segment or locus subject to meiotic drive, will be incorporated into the population when $2W_{01}k - 1 > 0$, as proposed by Sandler and Novitski (1957).

When $\Delta q = 0$ (i.e. at equilibrium), $q = \hat{q}$ in equation III. Hence the frequency of the driven element is equal to $\frac{2W_{01}k - 1}{2W_{01} - W_{00} - 1}$ as in equation IV and must lie between 0 and 1.

A driven element, under certain circumstances, can become fixed (i.e. reaching a frequency of 1) in the population. Here, the driven element can become fixed under three conditions

(1) In equation III, when $2W_{01} - W_{00} - 1 > 0$, the equilibrium is stable since Δq is +ve when $q < \hat{q}$ and -ve when $q > \hat{q}$. Stable equilibrium is attained, therefore, when $2W_{01} - W_{00} - 1 > 2kW_{01} - 1 > 0$, or

$$1 - \frac{W_{00}}{2W_{01}} > k > \frac{1}{2W_{01}}. \quad \text{When } k \geq 1 - \frac{W_{00}}{2W_{01}} > \frac{1}{2W_{01}}$$

i.e. $k > 2W_{01} - W_{00} - 1 > 0$, Δq is +ve for any value of $q < 1$.

Fixation of the driven element will take place when this condition is satisfied.

(ii) The driven element can also be fixed under unstable equilibrium

i.e. when $2W_{01} - W_{00} - 1 < 0$. The conditions for this are the

reverse for stable equilibrium, hence $2W_{01} - W_{00} - 1 < 2kW_{01} - 1 < 0$,

or $1 - \frac{W_{00}}{2W_{01}} < k < \frac{1}{2W_{01}}$. Under these conditions, the driven element

A_0 , will become fixed when its initial frequency $q_0 > \hat{q}$. The element will be lost, however, if $q_0 < \hat{q}$.

(iii) Fixation of A_0 will also occur when $2W_{01} - W_{00} - 1 = 0$.

Here k must be greater than $\frac{1}{2W_{01}}$ ($= 1 - \frac{W_{00}}{2W_{01}}$). The driven element will be lost however, if $k < \frac{1}{2W_{01}}$ ($= 1 - \frac{W_{00}}{2W_{01}}$).

Under random mating conditions, the effect of a driven element which is not associated with beneficial genes, is to reduce the mean fitness of the population. From equation 1,

$$\begin{aligned} \bar{W} &= q^2 W_{00} + 2q(1-q)W_{01} + (1-q)^2 \\ \frac{d\bar{W}}{dq} &= 2 \left[(W_{00}q - 2W_{01}q + q) + (W_{01} - 1) \right] \\ &= 2 \left[-q(2W_{01} - W_{00} - 1) + (W_{01} - 1) \right] \end{aligned}$$

for q to be maximum,

$$\frac{d\bar{W}}{dq} = 0$$

$$\text{i.e. } -q_{\max}(2W_{01} - W_{00} - 1) + (W_{01} - 1) = 0$$

$$\text{hence } q_{\max} = \frac{W_{01} - 1}{2W_{01} - W_{00} - 1}$$

Thus q_{\max} is only equal to \hat{q} (as in equation IV) when $k = \frac{1}{2}$. For any value of $k > \frac{1}{2}$ i.e. when under meiotic drive condition, q_{\max} is always smaller than \hat{q} .

Laboratory stocks of SD show that the value of K is fairly consistent at approximately 0.99. This high k value is not, however, maintained in wild populations of SD (Sandler et al 1959). The observed allele frequencies were within 2.3% and 3.3%. From population cages studies, the equilibrium frequency was about 10% (Miraizumi et al 1960). Hence, in the wild populations, there must be a special mechanism which suppresses this SD allele frequencies.

A driven element can become fixed in a population if its fitness is as high as or higher than that of its allele. When this situation exists, the role of meiotic drive is to accelerate the rate of increase of this driven element. The fitness of the whole population will increase if the driven element has a higher selective advantage than the other alleles. New mutants in wild populations, usually have lower fitnesses relative to already existing ones. Thus new instances of meiotic drive will normally have negative selection. The overall fitness of a population will hence be reduced, when the accumulation of the driven element takes place. Because most cases of meiotic drive are associated with a reduction in fitness it is generally thought that the phenomenon is detrimental to a population possessing it in nature. Meiotic drive, under certain conditions, can increase the fitness of an equilibrium population even though it is associated with reduced fitness. This is possible when the driven element is linked to some

beneficial genes. Initially, the driven element will increase the frequencies of the beneficial genes thus increasing the fitness of the population. A stage will come when the accumulation of the deleterious driven element has reached such a level that it is harmful to the population, the population fitness will fall. But if, at this point a mechanism has evolved which overcomes the deleterious effect of the driven element, then the beneficial genes would have been incorporated into the population more rapidly than they would have been in the absence of meiotic drive.

The association between abnormal segregation and beneficial genes is illustrated in the case of supernumerary chromosome Lilium callosum which exhibit preferential segregation in the embryo sac mother cells (Kimura and Kayano 1961). The supernumerary chromosome designated f_1 (meaning long telocentric fragment) when present in a single dose is believed to be deleterious and thus increases viabilities. With two f_1 's, the viability is about 70%, and with more than two f_1 's viability is virtually zero. The maintenance of these chromosomes in the population is effected merely by preferential segregation. In this system, modifiers which improve viabilities in combination with supernumerary chromosomes f_1 's are more advantageous when other factors are equal, than those which improve viabilities without supernumerary (B), since the B chromosomes have greater "transmission coefficient". Should such a system persist, the fitness of individuals with one B will become greater than that of those without B's. Consequently, the frequency of the supernumerary B carriers will increase and in conjunction with

this, the deleterious effect of the supernumerary chromosomes (i.e. reduction in viability) will be diminished.

A special case in which meiotic drive is advantageous is in an already existing balanced lethal. If meiotic drive causes an excess of one of the two alleles in the gametes of one sex and at the same time causes an excess of the alternative allele in the gametes of the other sex, then the zygotes produced will contain an excess of the heterozygotes. Thus the proportions of the two homozygotes are reduced. In this manner, lethal homozygotes are reduced in frequency and lethality will be thus diminished.

The difference in frequencies of SD between wild populations and populations in the laboratory is believed to be due to the association of SD with deleterious genes. In the wild populations, out of the 6 chromosome bearing SD recovered from a total of 183 second chromosomes, 5 were found to contain a recessive lethal. The same lethal genes are not found in laboratory stocks. Another factor which reduces SD in wild populations is that in the wild populations containing SD have accumulated insensitive SD^+ alleles. These alleles reduce the efficiency of the segregating mechanism and thus the equilibrium frequency of SD. It is possible that this SD^+ insensitive alleles become widespread in the population and when this happens the SD locus could be eliminated completely. Therefore, in wild populations of Drosophila which are devoid of SD, no such insensitive alleles are found because their presence would be

unnecessary. Such populations have been observed to exist in Japan.

The reduction in fitness associated with meiotic drive has been referred to as Distortional Load and its measure is the proportion of individuals which have to be eliminated to maintain the driven element in the population (Kimura, 1960). Distortional load is well illustrated in the case of "t-alleles" found in mice. This t allele, which is probably a system of multiple alleles, is a recessive gene on an autosomal chromosome and it causes complete sterility when homozygous. Due to preferential segregation of the t allele in heterozygous males (+/t), as high as 95% of the sperms functional during fertilisation may carry the t allele compared to only 5% which carry the normal allele, +. However, the frequency of t allele is only between 25% - 50% in the wild populations (Tutikawa, 1955; Dunn, 1955; Dunn and Suckling, 1956). Thus, although deleterious, the t allele is maintained in the population through abnormal segregation, in its favour.

If (i) $k (> 0.5)$ is the segregation proportion of t among the gametes produced by a heterozygous male, (ii) male heterozygotes and female heterozygotes have normal reproductive ability, then it can be shown (Kimura and Kayano, 1961) that the equilibrium proportion of t alleles among the male and female gametes are:

$$k = \frac{\sigma}{\sqrt{k(1-k)}} \quad \text{and} \quad \frac{1}{2k} \left[k + \frac{\eta}{\sqrt{k(1-k)}} \right]$$

The proportion of zygotes which are lethal will be the product of the above two functions, i.e.

$$= \frac{1}{2k} \left[k - \sqrt{k(1-k)} \right]^2$$

simplifying,

$$= \frac{1}{2} \left[1 - 2\sqrt{k(1-k)} \right]$$

This is the proportion of individuals which are eliminated from the population in each generation and hence a measure of distortional load, L_{SD} :

$$\text{therefore } L_{SD} = \frac{1}{2} \left[1 - 2\sqrt{k(1-k)} \right]$$

In the wild populations studied by Dunn (1957), k was found to lie between 0.89 and 0.99. From this, the distortional load is found to lie between 0.19 and 0.40. Hence, it is seen that in these populations, even disregarding possible effects of heterozygote advantage of t alleles, the load due to maintaining the deleterious t alleles, amounts to between 19 and 40 percent.

Meiotic drive, therefore is a medium through which the frequency of an element can increase in a population. Generally, meiotic drive is associated with a reduction in fitness and this resultant selection pressure keeps the driven element at a low frequency. However, when the element is associated with or linked to some beneficial genes, or with certain modifiers which neutralise its deleterious effects, the element can be significantly maintained in the population.

V. CONCLUSION

As exemplified by Zea mays, oogenesis and megasporogenesis result in the abortion of three of the four meiotic products; the remaining nucleus becomes the functional egg nucleus. In the maize, this becomes the basal megaspore and meiotic drive is brought about by the preferential segregation of the knobbed chromosome into the pole from which this megaspore arises. In spermatogenesis and microsporogenesis in which all four spermatids and microspores are functional (except in Drosophila in which some of the products of spermatogenesis are non-functional) distortion seems unlikely to occur except in those cases involving post meiotic death due to loss or lethality. As a result, meiotic drive should be expected to be more common in females than in males. However, this is not so. The only known case of meiotic drive in Drosophila female is the phenomenon referred to as "nonrandom disjunction". The fact that meiotic drive is common in Drosophila male is not surprising either. It should be noted that meiotic drive involves the unequal segregation of heterozygotes and in most of them, except in the case of SD, the segregation is between X and Y chromosomes. This is true in the case of sex-ratio, non-random segregation and recovery disrupter. In these circumstances, males can be considered as heterozygotes with respect to sex chromosomes. Females are homozygotes and for this reason, meiotic drive is rare in females. Although this explanation is not difficult to accept, the fact that only heterozygous males exhibit segregation distortion (SD), is still puzzling.

In the male Drosophila in which two kinds of poles, functional and nonfunctional exist, preferential segregation must therefore be preceded by some special orientation of chromosomes on the Metaphase I plate during meiosis. So far, there is no conclusive cytological evidence (except in maize) to suggest the existence of this special orientation. However, there is little doubt that meiotic drive is due to preferential segregation of chromosomes into functional poles.

In a number of species of both plants and animals, a special phenomenon resembling meiotic drive exists. Examples are those cases of "meiotic drive" of supernumerary (B) chromosomes. In Lilium callosum, (Kayano 1957, Kimura and Kayano, 1961), a supernumerary chromosome shows preferential segregation in the embryo-sac mother cells in such a way that in plants with a single B chromosome, it moves to the micropylar side in about 80% of the cases to be included in the egg cells. This phenomenon has not been observed in the male Lilium. Jackson and Cheung (1967) found a unique mode of segregation of chromosomes. They found that in the grasshopper Phaulacridium vittatum, the supernumerary chromosome and an X chromosome go to the same pole at a frequency of about 30 percent. This leads to an unequal frequency of supernumerary chromosome carriers in the two sexes (see Section Two of this thesis).

In chapter IV, it was shown that under certain conditions, a driven element could be incorporated, or could increase in frequency or even become fixed in a population. High frequency of such an element is very rare in nature, let alone its fixation. It is thus

speculated that there are other forces acting in natural populations whose interplay with meiotic drive proceeds in such a way that the latter's effectiveness is checked. A question arises, is meiotic drive an advantage or a disadvantage to the organisms exhibiting it? It seems that it would be a disadvantage since it is generally associated with a reduction in fitness. It only becomes an advantage when the driven element is associated with some beneficial genes, and this is a rare situation. Unfortunately, meiotic drive itself is a rare phenomenon and this makes the question regarding its significance more difficult to answer. Those examples which have so far been studied suggest that meiotic drive is a recent phenomenon. It could also be that its significant evolutionary consequence has yet to be effected.

PART II

RESEARCH: EFFECT OF TEMPERATURE ON MEIOTIC DRIVE

I. INTRODUCTION

Most cases of meiotic drive are most effective at the optimum temperature for growth and development of the organism exhibiting it. For Drosophila species, this optimum temperature lies in the region of 25°C. In a number of cases the intensity of meiotic drive has been known to be affected by culture at higher or lower temperatures than the optimum. Zimmering (1963) showed that in the case of non-random segregation, the frequencies of gametic types produced by $sc^4 - sc^8/Y$ males could be considerably changed if the males were raised at a lower temperature as at 18°C. Billet (1967) reported that both lowering and increasing of temperature decreased the amount of segregation distortion and that the effect was more pronounced in a marked line of SD. Although the results obtained by Billet were statistically not significant, they did show a trend of change due to the effect of temperature. Recovery disrupter in Drosophila has also been shown to be temperature sensitive. Erickson and Hank (1961) showed that the high recovery rate of X-chromosome could be nullified by lowering the temperature to 18°C. They also found that the stage in the life cycle which was sensitive to this change was in the process of spermatogenesis.

The effect of temperature on sex-ratio has been studied as early as 1942 in D. pseudoobscura by Darlington and Dobzhansky who reported

that the proportion of males dropped when the temperature of development was lowered. Faulhaber (1967) reported similar results in D. simulans. In the mosquito A. aegypti, temperature does not affect the expression of sex-ratio (Mickey 1965).

The primary aim of this work is to investigate the effect of temperature on two cases of meiotic drive, viz., Segregation Distortion and Nonrandom Segregation in D. melanogaster.

II. MATERIALS AND METHOD

Stocks:

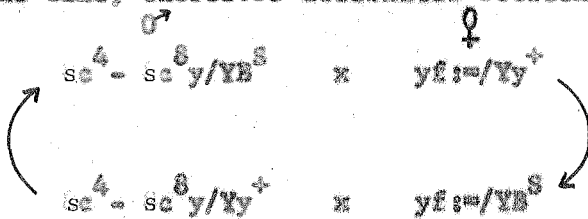
(i) Segregation-distortion.

Two stocks were used a) Stock heterozygous for SD-72 chromosome. This stock is also heterozygous for cn and bw. Hence, genotype of male heterozygous for SD is $\frac{SD}{+} \frac{cn}{+} \frac{bw}{+}$

b) Stock heterozygous for SD-72 bw chromosome. The second stock is different from the previous one in that it is homozygous for bw i.e. the SD-bearing chromosome is labelled with bw i.e. $\frac{SD}{+} \frac{cn}{+} \frac{bw}{bw}$.

(ii) Non-random Segregation.

The stock used was In(1) $sc^4 - sc^8 y/YB^S \times yf:=/Yy^+$. This cross produced disjunctional and non-disjunctional offsprings, and the stock was maintained by selecting two of the disjunctional offsprings viz., In(1) $sc^4 - sc^8 y/Yy^+$ males and $yf:=/YB^S$ females. The line, therefore alternated between these two generations i.e.



(iii) Test females.

a. Stock homozygous for cn and bw which were sensitive to SD action. Hence, female genotype $\frac{+}{+} \frac{cn}{cn} \frac{bw}{bw}$.

b. Yellow body strain, homozygous y/y (free-X females).

Food medium

The composition of the food medium used in all crosses was that of Whitten (1965). It consisted of,

- 90 ml. treacle
- 550 ml. of water
- 7.5 gm. agar (OXOID agar no. 3)
- 45 gm. compressed live baker's yeast
(Saccharomyces cerevisiae)
- 40 gm. Semolina
- 2.0 ml. propionic acid

This quantity of food was sufficient to fill about 50,4 x 1 inch tubes with about 3/4 inch of medium. Esterfoam bungs were used as tube stoppers.

In all crosses, except specifically stated, four males were mated to five females. All cultures in 18°C and 23°C temperature controlled ovens were continuously illuminated with fluorescent lamps.

III. SEGREGATION DISTORTION

Six tubes containing $\frac{SD}{+} \frac{+}{cn} \frac{+}{bw}$ males which had been raised for several generations at 25°C and $\frac{+}{+} \frac{cn}{cn} \frac{bw}{bw}$ virgin females were maintained at these temperatures 18°C, 25°C and 28°C for three successive generations. Flies were removed and counted at fairly regular intervals after first emergence. A similar experiment was also conducted for the marked stock i.e. for $\frac{SD}{+} \frac{+}{cn} \frac{bw}{bw}$ males. Results are shown in figures 1 and 2.

From the results, it is seen that both increasing and lowering of temperatures did not alter the value of k for either SD stocks. In the unlabelled SD chromosome stock, k was constant at 1.00 in all three generations at all three temperatures, showing no effect of temperature on segregation distortion. In the case of the bw labelled stock, k was insignificantly decreased from 1.00 to 0.996 in the first generation (G1) at 18°C and 28°C. But in the succeeding generations, k was again consistent at 1.00.

The above results are therefore not in agreement with those reported by Billet (1967). It should be emphasised that the results reported by Billet for the unlabelled stock were statistically not significant. Despite this however, the results did show a trend due to a change in temperature. The stocks used in this investigation were much more stable and this stability to temperature change could be due to the stabilising modifier $St(SD)$.

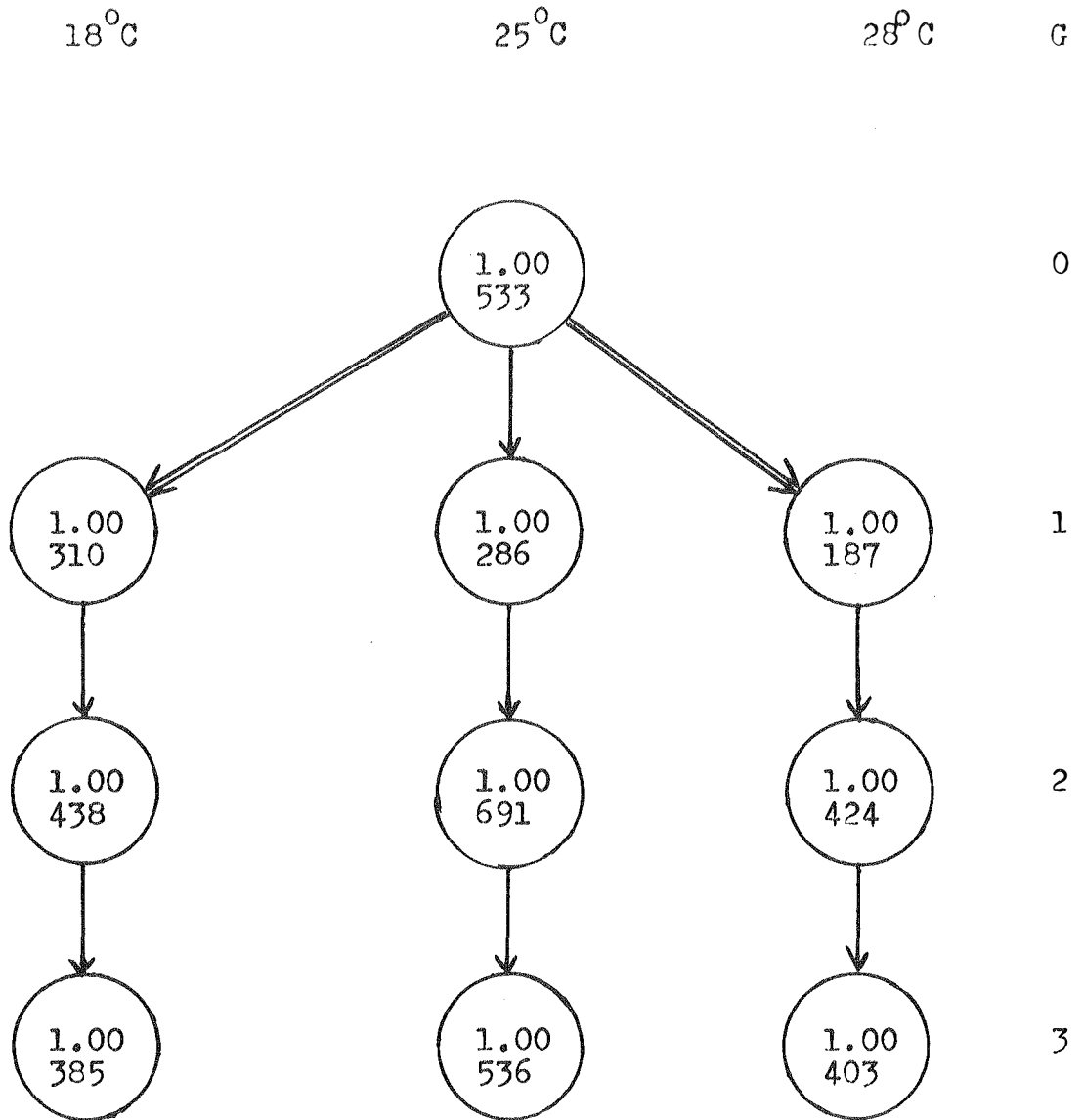


Figure 1. Effect of temperature on SD. All crosses are between $\frac{SD + +}{+ cn bw}$ males and $\frac{+ cn bw}{+ cn bw}$ females. Upper figures are values for k and lower figures are values for N (= total progeny).

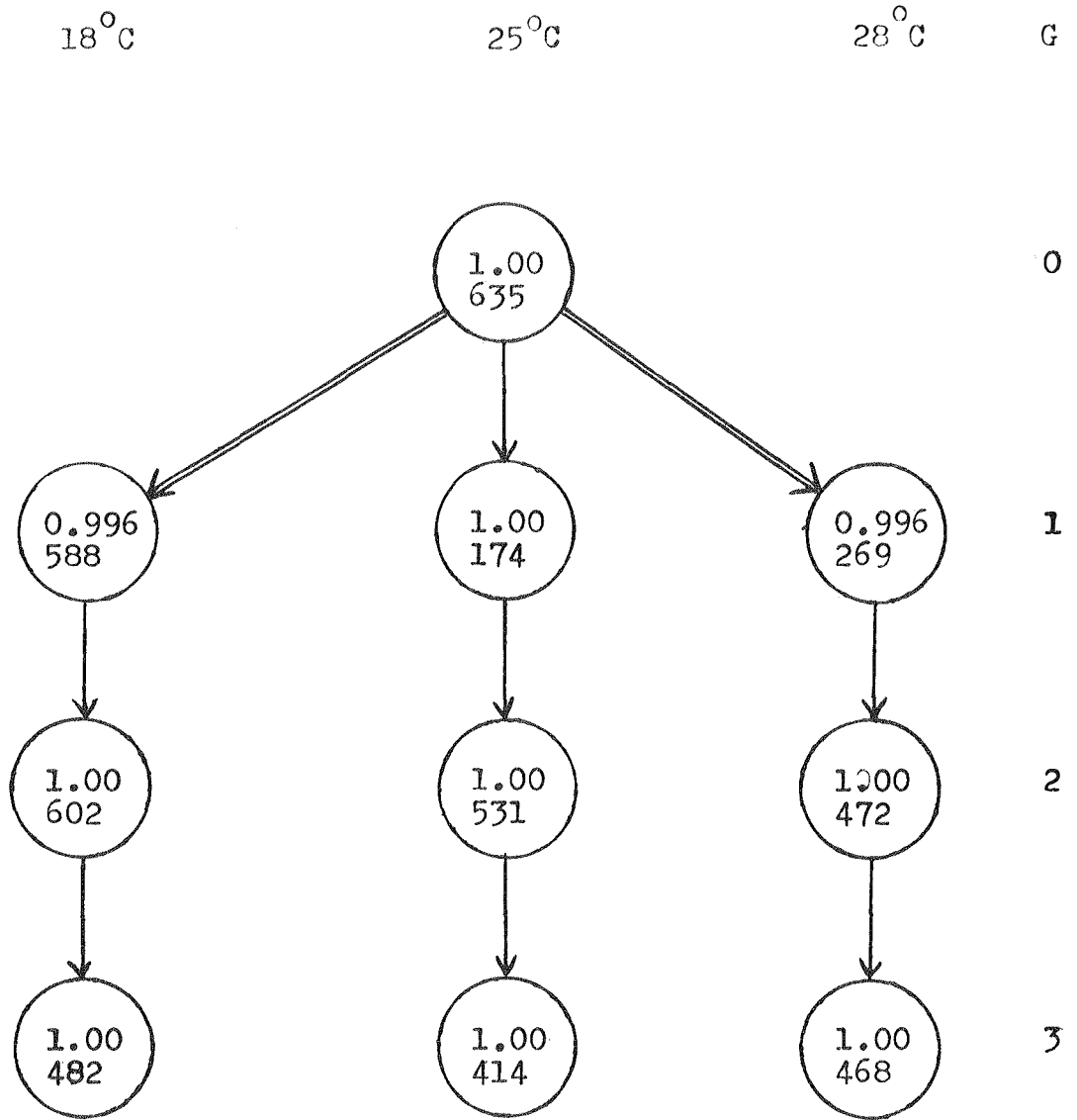


Figure 2. Effect of temperature on SD. All crosses are between males carrying SD-bearing chromosome labelled with bw, $\frac{SD + bbw}{+ cn bw}$ and $\frac{+ cn bw}{+ cn bw}$ females.

IV. DEVIAANT SEX-RATIO IN ASSOCIATION WITH SD

In all crosses between $\frac{SD}{+} \frac{+}{cn\ bw}$ males and $\frac{cn}{cn} \frac{bw}{bw}$ females, there were more males found among the SD progeny than females. This deviation in individual progenies bordered on significance and was consistent between progenies. This preliminary observation supports the report of Hiraizumi and Nakazima (1967). Hiraizumi and Nakazima also reported that more females were found among the small cn bw progeny. However, this point was not observed in the stock used since the percentage of cn bw among the offsprings was negligible.

The sex-ratio associated with SD was found to be insensitive to temperature change, see Table 1. The temperature/sex-ratio interaction χ^2_{11} from the 2X2 contingency has a value of 0.17 showing no evidence of interaction ($0.7 > P > 0.5$). The individual progenies raised at 25°C and 18°C show a similar disturbance of the sex-ratio:- 53.2% and 54.6% of males respectively. The deviation from 1:1 ratio borders on significance in each ($P > 0.5$). The combined sex-ratio disturbance, however, gives a deviation χ^2_{11} of 5.55 indicating a significant deviation from 1:1 ($0.02 > P > 0.01$). Thus the bordering significance of sex-ratio deviation in the preliminary observations was due to the small number of individuals among the progeny.

From the above results, it appears therefore that like segregation distortion, the deviant sex-ratio associated with it is also temperature insensitive. This point gives support to the model proposed by Hiraizumi and Nakazima who proposed that there existed a direct

TABLE 1

Effect of Temperature on Sex-Ratio associated with SD

Temp.	♂	♀	Total	χ^2	P
25°	335	294	629	2.67	0.1 > P > 0.05 Bordering significance
18°	195	162	357	3.05	0.1 > P > 0.05 Bordering significance
Total	530	456	986	5.55	0.02 > P > 0.01**

$$\begin{aligned} \text{Interaction } \chi_{[1]}^2 &= \left[\frac{(195 \times 294) - (335 \times 162)}{629 \times 357 \times 530 \times 456} \right]^2 986 \\ &= 0.17 \\ &0.7 > P > 0.5 \quad \text{N.S.} \end{aligned}$$

or alternatively,

$$\begin{aligned} \text{Heterogeneity } \chi_{[1]}^2 &= (2.67 + 3.05) - 5.55 \\ &= 0.17 \end{aligned}$$

$$\begin{aligned} \text{Deviation } \chi^2 &= 5.55 \\ &0.02 > P > 0.01^{**} \end{aligned}$$

relationship between SD and sex-ratio (see page 17). Since SD is not affected by temperature, on the basis of this model, sex-ratio is also not expected to be affected by temperature changes. The above results showed that this is so.

V. NONRANDOM SEGREGATION

In the maintenance of the stock, the two alternating parents, $sc^4 - sc^8 y/Yy^+ \sigma \times yf := /YB^S Q$, produced eight types of offspring, only half of which were viable. An accurate estimation of X/Y and O/XY ratios was therefore not possible. To get reliable figures, the males were crossed to free-X females $y//y$. This gave only four classes of offsprings, two of which were disjunctional and the other two were non-disjunctional, and all were viable. Estimation of X/Y and O/XY was made directly through the corresponding ratios among the offsprings.

(1) Sensitivity to temperature change

A preliminary experiment showed that males raised at the optimum temperature at $25^{\circ}C$, produced gametes which consisted of twice as many X as there were Y gametes, and nullo gametes were in great excess over XY gametes ($X/Y \approx 2$ and $O/XY \approx \infty$). The percentage of X and Y gametes i.e. of disjunctional gametes, was about 80% showing a high percentage of non-disjunctional gametes of about 20%. This generation is made as the first generation (G1) in the whole temperature experiment scheme, (see Figures 3 and 4).

To study the effect of temperature on nonrandom segregation, six tubes containing $sc^4 - sc^8 y/YB^S$ or $sc^4 - sc^8 y/Yy^+$ males which had been raised for several generations at $25^{\circ}C$ and virgin $y//y$ females, were maintained at three temperatures, $18^{\circ}C$, $25^{\circ}C$ and $28^{\circ}C$ for three successive generations. Flies were removed and counted at regular intervals after first emergence.

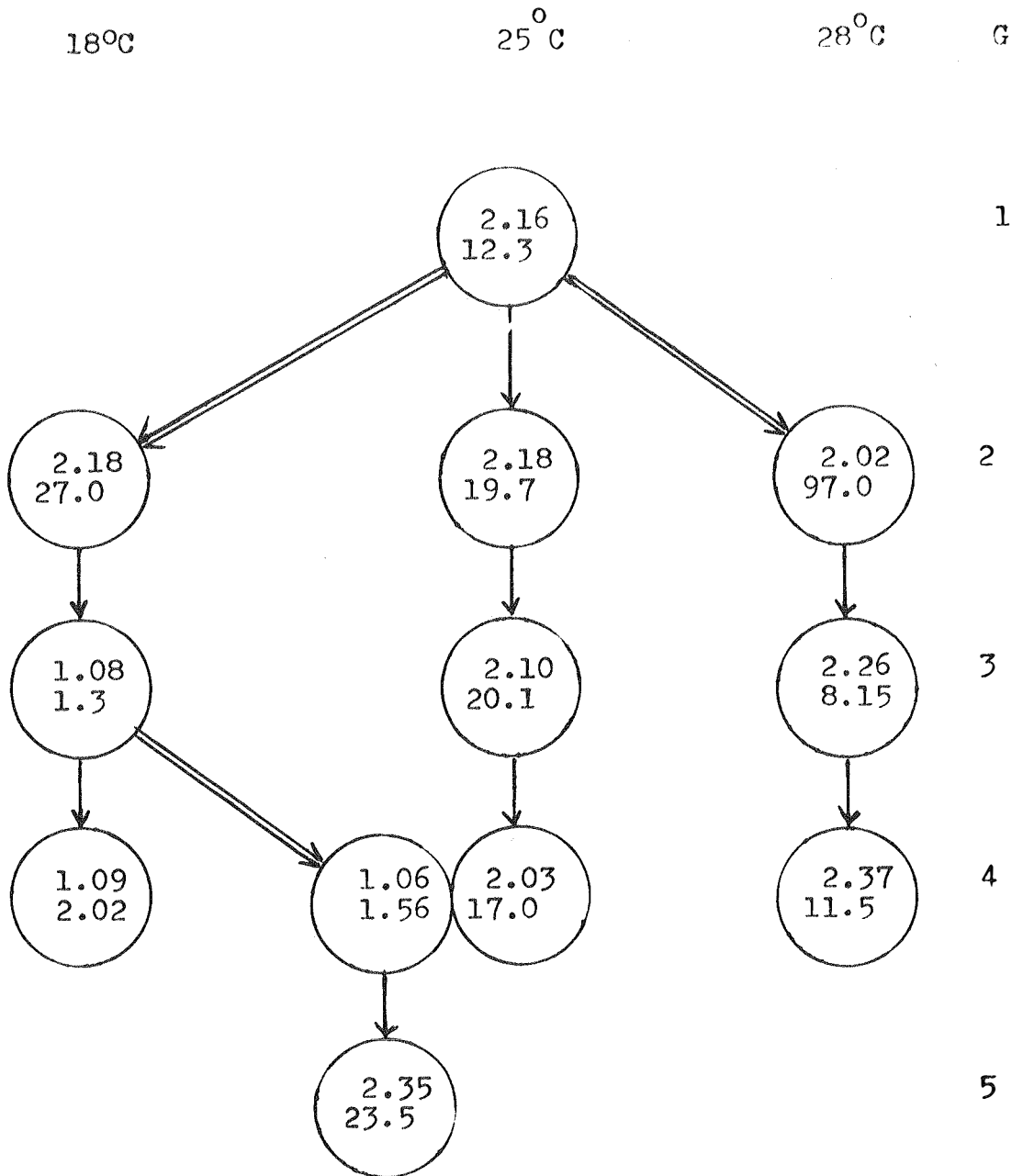


Figure 3. Effect of temperature on Nonrandom Segregation In(i)sc⁴-sc⁸. Upper figures are X/Y ratios and lower figures are O/XY ratios among the progeny of crosses between sc⁴-sc⁸/YB^s males and y//y females (free-X) in five successive generations.

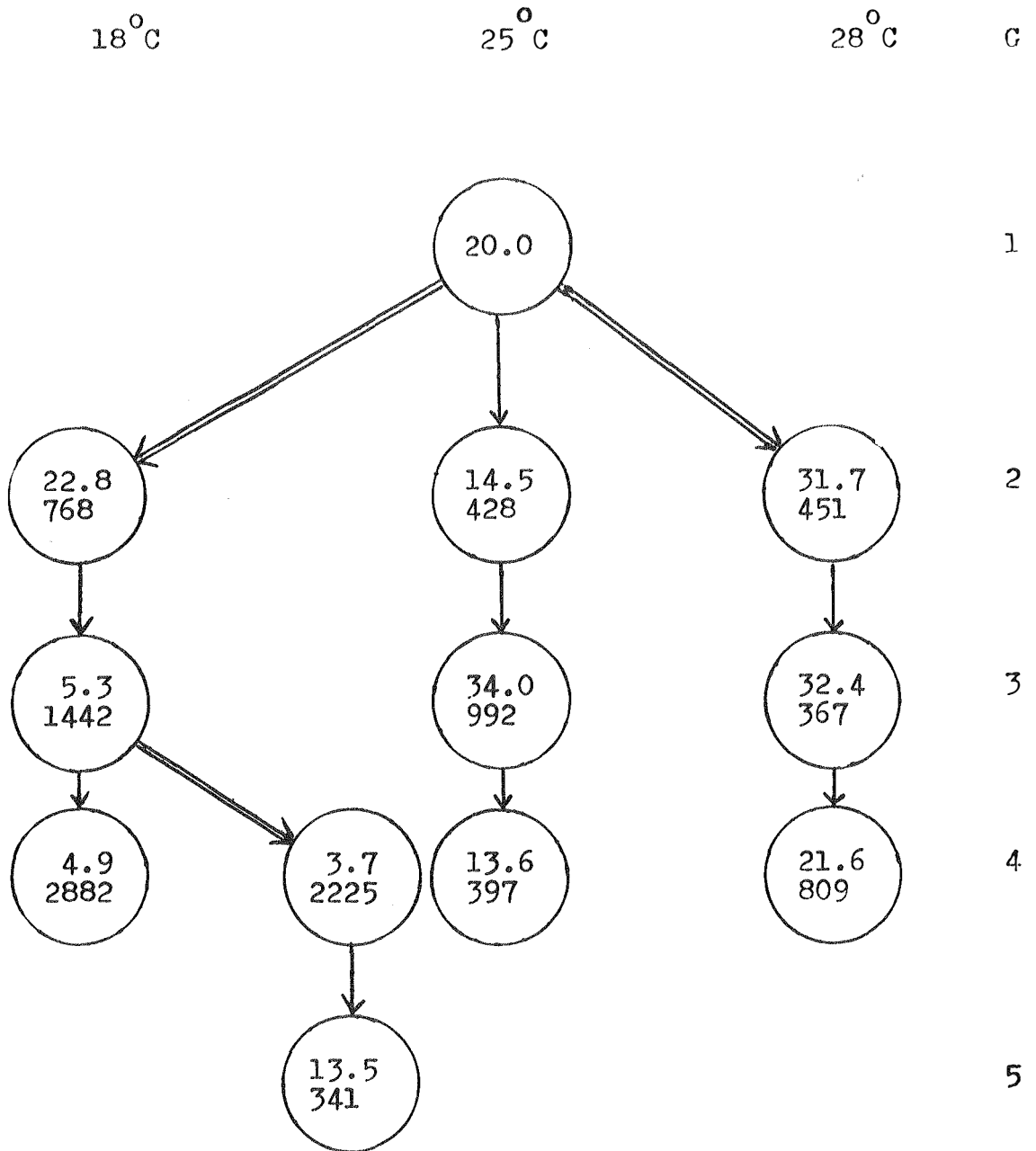


Figure 4. Effect of temperature on the percentage of nondisjunctional gametes of sc^4-sc^8/Y males which showed Nonrandom Segregation. Lower figures = N.

In the second generation, there was no significant change in either ratio of X/Y or O/XY (Table 2). At the control temperature (25°C), X/Y = 2.18 and O/XY = 19.7. Although O/XY had increased from 12.3 in G1 to 19.7 in G2, the fact that nullo gametes were in great excess over XY gametes was evident. At the lower temperature at 18°C, X/Y = 2.18 showing no significant change from the X/Y ratio at 25°C at G2 $\left[\chi^2 = 3 \times 10^{-6}, P > 0.999, \text{N.S.} \right]$ and O/XY = 27.0 also showing no significant change from the corresponding value at 25°C at G2 $\left[\chi^2 = 0.193, 0.7 > P > 0.5, \text{N.S.} \right]$. Similarly, at the higher temperature at 28°C, X/Y = 2.02 and O/XY = 97, there was no significant change although the ratio O/XY had increased from 12.3 to 97.0

$$\left[\chi^2_{\text{X/Y}} = 0.244, 0.7 > P > 0.5, \text{N.S. and } \chi^2_{\text{O/XY}} = 2.27, 0.2 > P > 0.1, \text{N.S.} \right]$$

In the third generation at 25°C (Table 3), X/Y = 2.10 and O/XY = 20.1 showing no significant difference from the corresponding values in G2 $\left[\chi^2_{\text{X/Y}} = 0.68, 0.8 > P > 0.7, \text{N.S. and } \chi^2_{\text{O/XY}} = 0.0089, 0.95 > P > 0.9, \text{N.S.} \right]$. At 28°C, X/Y = 2.26 and O/XY = 8.15 $\left[\chi^2_{\text{X/Y}} = 0.417, 0.7 > P > 0.5, \text{N.S. and } \chi^2_{\text{O/XY}} = 8.74, 0.01 > P > 0.001^{***} \right]$.

Here again, there was no significant deviation except in the case of O/XY. This was simply due to the small number of XY class recovered in G2. Nevertheless, nullo gametes were still in great excess over XY gametes. However, at 18°C, there was a significant change in gametic frequencies of males; X/Y dropped from 2.18 in G2 to 1.08

$$\left[\chi^2_{\text{X/Y}} = 45.29, P < 0.001^{***} \right] \text{ and O/XY dropped from 27.0 to 1.3 } \left[\chi^2_{\text{O/XY}} = 6.19, 0.02 > P > 0.01^{**} \right]. \text{ At the lower temperature,}$$

TABLE 2

Progeny of crosses between sc^4 sc^8/Y^s males and y/y females (free-X) at three different temperatures, in generation 2. N = total progeny. Flies were scored at different emergence.

16°C

25°C

28°C

Ganetes formed by male.

X	Y	O	XY	X	Y	O	XY	X	Y	O	XY
42	20	15	1	59	12	5	1	46	21	18	1
84	27	37	2	42	16	7	1	58	22	11	0
97	39	31	1	25	10	6	0	35	31	27	0
39	16	11	0	26	8	7	0	56	30	35	0
35	27	24	1	46	24	11	0	41	13	6	0
72	37	36	1	53	45	23	1				
14	7	4	0								
8	6	4	0								
391	179	162	6	251	115	59	3	236	117	97	1

N = 738

N = 428

N = 451

$X/Y = 2.18$
 $O/XY = 27.0$

$X/Y = 2.18$
 $O/XY = 19.67$

$X/Y = 2.02$
 $O/XY = 97.0$

$\frac{X+Y}{N} = 77.2\%$

$\frac{X+Y}{N} = 85.5\%$

$\frac{X+Y}{N} = 78.3\%$

$\frac{O+XY}{N} = 22.8\%$

$\frac{O+XY}{N} = 14.5\%$

$\frac{O+XY}{N} = 21.7\%$

TABLE 3

Progeny of crosses between sc^{48}/Yy^{+} males and \bar{y}/\bar{y} females (free-X)
at three different temperatures, in generation 3. N = total progeny.

18°C

25°C

28°C

Gametes formed by male.

X	Y	O	XY	X	Y	O	XY	X	Y	O	XY
107	86	11	6	91	53	15	0	25	12	17	2
39	39	2	3	82	35	26	6	11	3	2	0
81	65	3	3	66	32	31	3	28	12	15	3
65	76	4	4	9	4	8	0	73	32	40	7
48	55	5	5	70	26	92	2	17	9	12	0
213	186	17	9	89	47	122	3	10	4	9	0
78	58	0	2	24	8	18	2	8	4	11	1
79	91	0	1	13	6	9	0				
710	656	43	33	444	211	321	16	172	76	106	13

N = 1442

N = 992

N = 367

X/Y = 1.08

X/Y = 2.10

X/Y = 2.26

O/XY = 1.30

O/XY = 20.1

O/XY = 8.15

$\frac{X+Y}{N} = 94.7\%$

$\frac{X+Y}{N} = 66.0\%$

$\frac{X+Y}{N} = 67.6\%$

$\frac{O+XY}{N} = 5.3\%$

$\frac{O+XY}{N} = 34.0\%$

$\frac{O+XY}{N} = 32.4\%$

therefore, the frequencies of X and Y and also of O and XY (i.e. the frequencies of gametes within disjunctional and non-disjunctional classes respectively) approached equality.

This equality in frequencies of X and Y and also of O and XY at 18°C persisted in the following generation (G4), see Table 4. Here $X/Y = 1.09$ and $O/XY = 2.02$ $\left[\chi^2_{X/Y} = 0.0016, P > 0.95 \text{ N.S.} \right]$ and $\chi^2_{O/XY} = 2.27, 0.2 > P > 0.1, \text{ N.S.} \left[\right]$ showing no real difference from the corresponding values in the previous generation, G3.

The inequality of the frequency of disjunctional and non-disjunctional gametes at 25°C and 28°C was also maintained in G4. At 25°C, $X/Y = 2.03$ and $O/XY = 17.0$ $\left[\chi^2_{X/Y} = 0.07, 0.8 > P > 0.7, \text{ N.S.} \right]$ and $\chi^2_{O/XY} = 0.065, 0.8 > P > 0.7, \text{ N.S.} \left[\right]$ and at 28°C, $X/Y = 2.37$ and $O/XY = 11.5$ $\left[\chi^2_{X/Y} = 0.084, 0.8 > P > 0.7, \text{ N.S.} \right]$ and $\chi^2_{O/XY} = 0.73, 0.5 > P > 0.3, \text{ N.S.} \left[\right]$.

Lowering of temperature from the optimum growth and development temperature at 25°C to 18°C therefore, decreased the effectiveness of meiotic drive in Nonrandom Segregation - In (1) sc^4-sc^8 . Increasing of temperature from 25°C to 28°C produced no similar change in meiotic drive. It should be noted that the ratio X/Y in all four generations at the control temperature remained constant ($X/Y \approx 2$). Also, in all four generations at 25°C, nullo gametes were in great excess over XY gametes. The change in X/Y and O/XY from G2 to G3 at 18°C was therefore, due to temperature effect and not due to other factors such as differences in line stability.

TABLE 4

Progeny of crosses between sc^{-sc}/YB^s males and y/y females (free-X) at three different temperatures, in generation 4. N = total progeny.

18°C				25°C				28°C			
Gametes formed by male.											
X	Y	0	XY	X	Y	0	XY	X	Y	0	XY
210	177	11	4	37	17	3	0	58	27	27	1
113	118	5	0	43	26	10	3	72	22	22	4
128	117	10	6	15	10	7	0	81	35	20	1
192	106	2	3	37	19	10	0	54	23	17	3
233	213	7	9	24	11	6	0	45	19	20	0
202	177	6	9	13	12	4	0	61	25	17	1
133	94	43	9	48	15	9	0	75	37	38	4
303	310	11	7	12	3	2	0	—	—	—	—
1426	1314	95	47	229	113	51	3	446	188	161	14
N = 2382				N = 397				N = 809			
X/Y = 1.09				X/Y = 2.03				X/Y = 2.37			
0/XY = 2.02				0/XY = 17.0				0/XY = 11.5			
$\frac{X+Y}{N} = 95.1\%$				$\frac{X+Y}{N} = 86.36\%$				$\frac{X+Y}{N} = 78.37\%$			
$\frac{0+XY}{N} = 4.9\%$				$\frac{0+XY}{N} = 13.64\%$				$\frac{0+XY}{N} = 21.63\%$			

An experiment was done to determine whether or not the effect of temperature was reversible. Six tubes containing sc^4-sc^8/YB^S males raised at $18^\circ C$ and virgin $y//y$ females, were kept at $25^\circ C$. The results are shown in Table 5. In the first generation, corresponding to G4 in figure 3, $X/Y = 1.06$ and $O/KY = 1.56$ which did not differ significantly from the corresponding values in G3 at $18^\circ C$ [$\chi^2_{X/Y} = 0.68, 0.8 > P > 0.7, N.S.$ and $\chi^2_{O/KY} = 0.0089, 0.95 > P > 0.9, N.S.$]. However, in the following generation (G5), the frequencies of gametes within disjunctional and nondisjunctional classes were typical of meiotic drive in Nonrandom Segregation at optimum temperature with $X/Y = 2.35$ and $O/KY = 23.5$ [$\chi^2_{X/Y} = 36.64, P < 0.001^{***}$ and $\chi^2_{O/KY} = 19.49, P < 0.001^{***}$]. Thus, the effect of temperature on Nonrandom Segregation was reversible.

An interesting point was observed regarding the percentage of non-disjunctional gametes formed by sc^4-sc^8/Y males. In conjunction with X/Y and O/KY ratios, the percentage of non-disjunctional gametes were also affected by temperature change. When X/Y and O/KY approached equality as in G3 at $18^\circ C$, it was found that the percentage of non-disjunctional gametes i.e. $\frac{X+Y}{N}$ decreased significantly from about 15% - 30%, to nearly 3% (Figure 4). Thus, lowering the temperature from $25^\circ C$ to $18^\circ C$ decreased the percentage of non-disjunctional gametes, or alternatively, increased the percentage of disjunctional gametes. This effect, like that of temperature on X/Y and O/KY was also reversible i.e. increasing the temperature from $18^\circ C$ to $25^\circ C$, increased the percentage of non-disjunctional gametes, or alternatively,

TABLE 5

Progeny of crosses between sc^4-sc^8/Y males (which had been raised at $18^\circ C$) and $y//y$ females (free $-X$) and culture transferred to $25^\circ C$, in generations 4 and 5.

Gametes formed by male

	<u>X</u>	<u>Y</u>	<u>O</u>	<u>XY</u>	<u>N</u>	<u>X/Y</u>	<u>O/XY</u>	<u>$\frac{X+Y}{N}$</u>
G4	297	280	25	12				
	351	356	13	11				
	453	406	12	9				
	<hr/>	<hr/>	<hr/>	<hr/>				
	1101	1042	50	32	2225	1.06	1.56	96.3%
G5	46	24	9	1				
	47	16	10	1				
	78	24	16	0				
	24	16	10	0				
	10	7	2	0				
	<hr/>	<hr/>	<hr/>	<hr/>				
	205	87	47	2	341	2.35	23.5	86.5%

decreased the percentage of disjunctional gametes (Table 5).

Another interesting point observed was that, in Figures 3 and 4, the effect of temperature, both increasing and lowering, had a lag of one generation. This observation led to the belief that, there was a certain phase in the development of the sc^4-sc^8/Y males which was sensitive to this change in temperature, in consequence of which the ratios of X/Y and O/XY and also the percentage of disjunctional and non-disjunctional gametes were accordingly affected.

(11) Time of Temperature Sensitivity

The time of temperature sensitivity or the time of response to temperature change was determined by transferring males from a higher to a lower temperature and vice-versa and finding the frequencies of all four gametic types utilised in successive broods. Twenty sc^4-sc^8/YB^5 males raised at $25^{\circ}C$ which were between 0 and 2 days old were mated singly to five $y//y$ virgin females also of 0 to 2 days old. The twenty tubes were kept at $18^{\circ}C$. Every four days, the males were remated singly to further five virgin $y//y$ females in fresh tubes and kept at $18^{\circ}C$. The five original $y//y$ females were retained in the old culture tubes and later discarded when larvae appeared. Seven broods were maintained and the results are shown in Tables 6, 7 and 8.

From the twenty males employed, only three (nos. 7, 14 and 15) produced offsprings in at least six broods. Six males failed to produce any offspring in all seven broods. Ratio of X/Y among the offspring of the three males are plotted against broods in Figure 5 and the pooled data are shown in Figure 6.

TABLE 6

Ratios of X/Y in successive 4-day broods among the offsprings of sc^4-sc^8/YB^8 males transferred from $25^{\circ}C$ to $18^{\circ}C$ at the beginning of Brood I.

Male	Brood						
	i	ii	iii	iv	v	vi	vii
1	1.67						
3	0.67						
6							
7	1.67	2.25	1.89	1.57		1.78	1.24
9	3.0						
10						0.67	1.64
11	1.5						
12	1.4				4.0		
13	2.0						
14	2.67		1.77	1.64	1.29	1.41	1.14
15	1.46	2.0	2.17	1.26	0.81	1.38	1.29
16	7.0						
18	2.0						
19	5.67	2.25			2.75		
mean	2.12	2.22	1.90	1.53	1.32	1.35	1.34

TABLE 7

Ratios of O/XY in successive 4-day broods among the offsprings of sc^4-sc^8/Y^S males transferred from $25^{\circ}C$ to $18^{\circ}C$ at the beginning of Brood I.

Male	i	ii	iii	Brood iv	v	vi	vii
1	0						
3	6						
6							
7	2	2	7.5	4.3		3	2.5
9	5						
10		20				1	1
11	3						
12	3				0		
13	4						
14	5		5.7	1.3	10	1	5.5
15	10	1	10.0	3.5	2	5	5.0
16	5						
18	6						
19	9				3		
mean	58.0	20.0	7.0	2.4	11.3	2.0	4.4

TABLE 8

Percent disjunctive offsprings in successive
4-day broods from sc^4-sc^8/YB^8 males transferred
from 25°C to 18°C at the beginning of Brood I.

Male	Brood						
	i	ii	iii	iv	v	vi	vii
1	100						
3	45						
6							
7	80	86.7	86.6	91.6		86.2	83.9
9	44						
10		86.3				78.9	97.4
11	74						
12	85				100		
13	77						
14	69		82.9	84.7	10	94.2	78.3
15	76	88.9	83.8	88.6	4	93.1	98.6
16	58						
18	25						
19	69				3		
mean	68.5	86.3	84.6	89.1	81.4	93.5	94.7
N	187	153	312	466	199	186	509

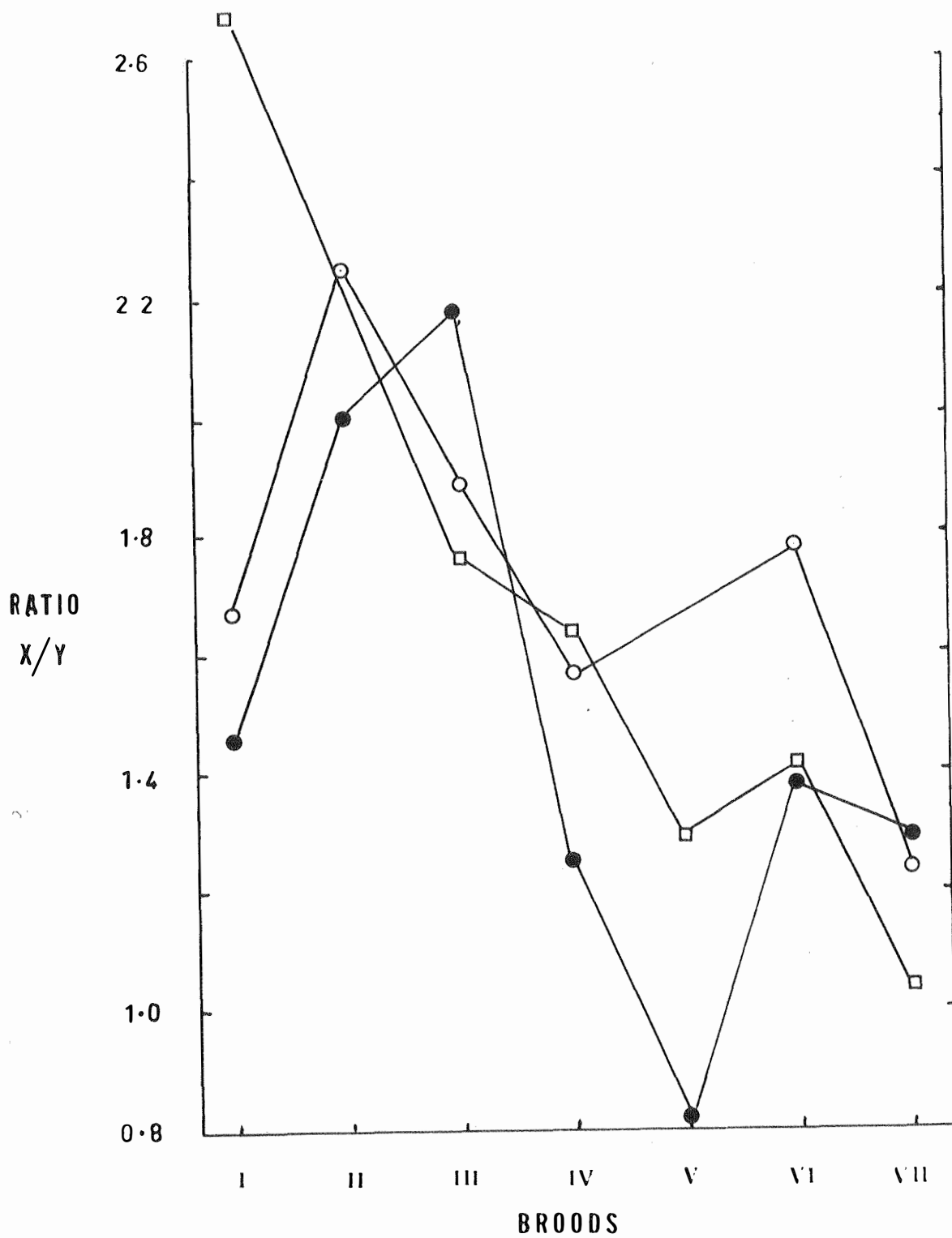


Figure 5. Change in ratios of X/Y in 4-day broods after transfer from 25°C to 12°C for three males no. 7, 14, 15.

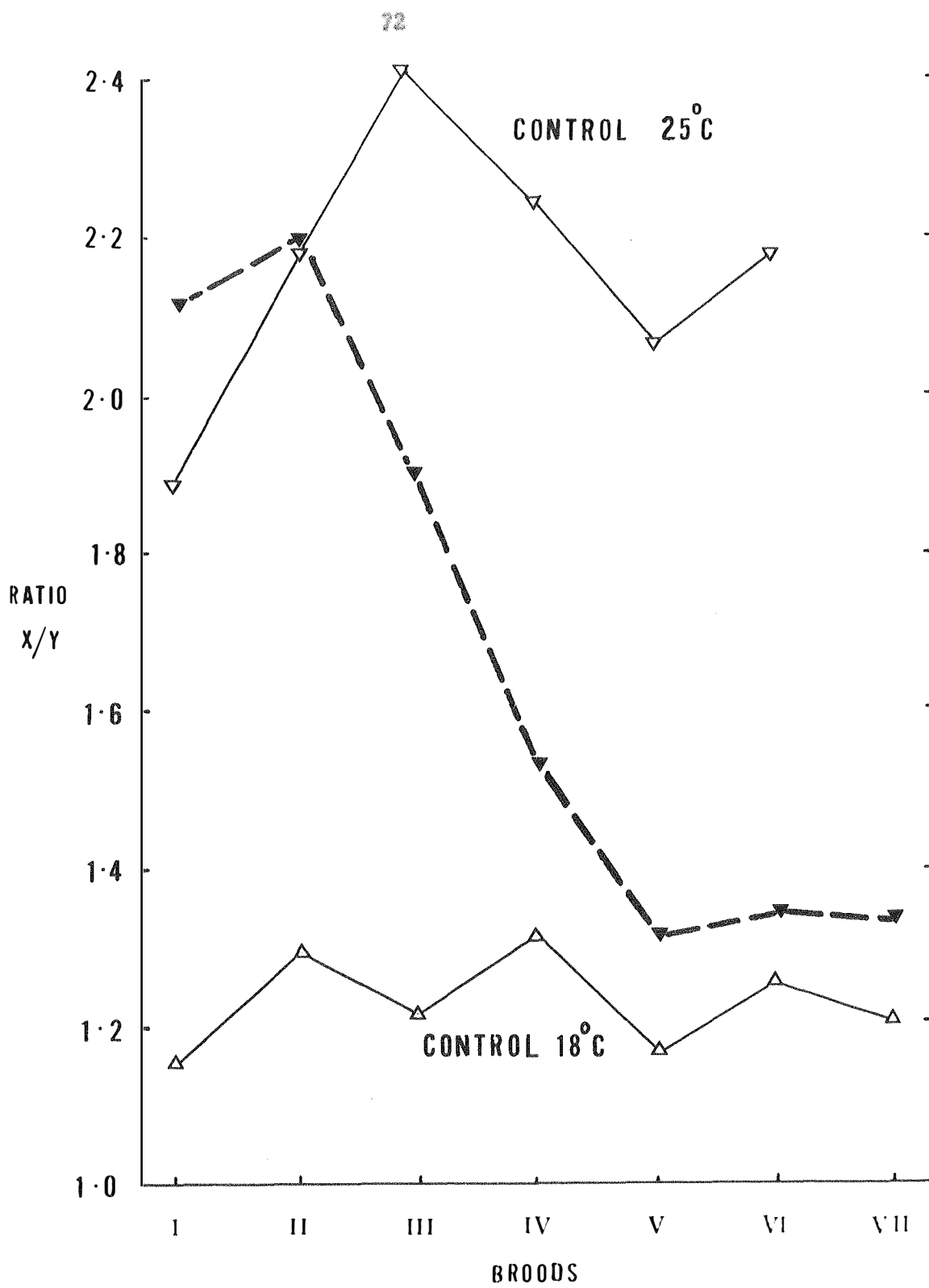


Figure 6. Change in mean ratios of X/Y in 4-day broods after transfer from 25°C to 18°C.

Control experiments were also conducted at 18°C with 4-day broods and at 25°C with 2-day broods. Results are given in Tables 9 and 10 respectively. Unfortunately in the control at 25°C, no reliable data were available for brood VII since the offspring produced in this brood were too small in number. Ratio of X/Y are also plotted together with temperature decreasing experiment data in Figure 6.

The ratios of X/Y at the control temperature at 18°C, fluctuated about a mean of 1.226 ± 0.058 for 95% confidence limit. In Brood I, $X/Y = 1.15$ which was slightly outside the lower limit of the level of 95% confidence. The value, however, increased to 1.29 in Brood II, which was slightly outside the upper limit of the 95% confidence level. The value of X/Y in Brood IV also showed significant deviation. The remaining X/Y ratios showed no significant differences at constant temperature.

At the higher control temperature at 25°C, the ratios of X/Y gave a mean of 2.16 ± 0.187 for 95% confidence level of significance. Except in the case of Brood III, there were no significant differences among X/Y ratios. Like the case at the lower temperature, it is reasonable to conclude that X/Y ratios at the higher constant temperature, remained constant. It is also reasonable to conclude that paternal age of parents had no effect on ratios of X/Y at both constant temperatures, 18°C and 25°C.

The time of sensitivity to an increase in temperature from 18°C

TABLE 9

Progeny of sc^4-sc^8/Y males in successive 4-day
broods at constant temperature control of $18^\circ C$.

Brood	Gametes formed by male				N	X/Y	O/XY	$\frac{X+Y}{N}$
	X	Y	O	XY				
i	196	170	16	11	393	1.15	1.45	93.1
ii	191	148	17	4	360	1.29	4.25	94.2
iii	181	150	31	7	369	1.21	4.43	89.7
iv	87	66	15	6	174	1.31	2.5	87.9
v	108	92	20	12	232	1.17	1.67	86.2
vi	195	156	34	19	404	1.25	1.79	86.9
vii	126	105	15	11	257	1.20	1.36	89.9

TABLE 10

Progeny of sc^4-sc^8/Y males in successive 2-day broods at constant temperature control of $25^\circ C$.

Gametes formed by male								
Brood	<u>X</u>	<u>Y</u>	<u>O</u>	<u>XY</u>	<u>N</u>	<u>X/Y</u>	<u>O/XY</u>	<u>X+Y</u> <u>N</u>
i	169	90	13	2	274	1.88	6.5	94.5
ii	24	11	9	0	44	2.18	∞	79.5
iii	70	29	15	0	114	2.41	∞	86.8
iv	94	42	26	3	165	2.24	12	82.4
v	107	52	31	3	193	2.06	6.2	82.4
vi	85	39	23	2	149	2.17	11.5	83.2
vii	no results available for this brood.							

to 25°C (the reverse experiment) was studied in a similar manner. However, in this increase temperature experiment, the brood period was two days. The change in ratios among the progeny of twelve sc^4-sc^8/YB^a males were followed. Results are shown in Tables 11, 12 and 13. As in the decrease temperature experiment, complete records of all male progeny were not available. Two males failed to produce offsprings while only four gave near complete record. The X/Y ratios of three males (nos. 6, 9 10) are plotted against broods in Figure 7 and the pooled data are shown in Figure 8.

TABLE 11

Ratios of X/Y in successive 2-day broods among the offsprings of sc^4-sc^6/YB^8 males transferred from $18^{\circ}C$ to $25^{\circ}C$ at the beginning of Brood I.

Male	Brood						
	i	ii	iii	iv	v	vi	vii
1	1.42	1.31	1.17	2.93			
2			1.75	1.50			
4	0.98		0.83	1.50	1.47		
5				1.36		1.29	
6	1.07	0.89	1.70	1.37	1.85	1.0	1.86
7				1.57		3.4	1.95
9	0.96	1.60	1.66	2.21	2.0	1.5	1.55
10	0.98	1.11	2.59	2.11			1.86
11		1.32	1.05	1.61			
12		0.87	1.15	1.20			
mean	1.05	1.19	1.30	1.55	1.64	1.68	1.79

TABLE 12

Ratios of O/XY in successive 2-day broods among the progeny of sc^4-sc^6/YB^6 males transferred from $18^\circ C$ to $25^\circ C$ at the beginning of Brood I.

Male	Brood						
	i	ii	iii	iv	v	vi	vii
1		1.0	1.0	3.0			
2	5.0		1.0	2.0			
4	2.0		3.0	6.0	13.0		
5				0.3		3.0	
6	1.0	11.0	5.0	0.4	18.0		14.0
7				4.5		3.0	5.0
9	0.6	4.8	9.5	10.0	3.0	3.0	8.0
10	1.0	3.0	1.0	3.0			9.0
11		5.0	6.0	1.5			
12		1.3	3.7	2.2			
mean	1.5	3.9	6.0	1.68	18.0	9.0	10.6

TABLE 13

Percent disjunctive offsprings in successive
2-day broods from sc^4-sc^8/YB^8 males transferred
from 18°C to 25°C at the beginning of Brood I.

Male	Brood						
	i	ii	iii	iv	v	vi	vii
1	%	94.9	98.4	88.7			
	n	39	64	71			
2	83.7		100.0	93.0			
	80		22	43			
4	97.9		95.5	93.0	83.2		
	97		67	86	94		
5				96.9		97.5	
				125		117	
6	91.1	86.0	81.8	77.3	67.8	100	85.1
	134	86	33	132	52	12	101
7				93.3		95.7	90.3
				165		69	62
9	95.7	83.9	85.6	81.8	66.6	90.9	81.4
	115	180	146	55	8	33	97
10	94.6	97.4	97.7	90.5			84.0
	111	154	43	42			119
11		96.4	91.9	93.7			
		166	87	159			
12		93.6	89.2	88.5			
		110	130	139			
mean	94.4	91.9	91.7	89.9	76.7	96.2	84.9
N	537	745	588	1011	326	234	378

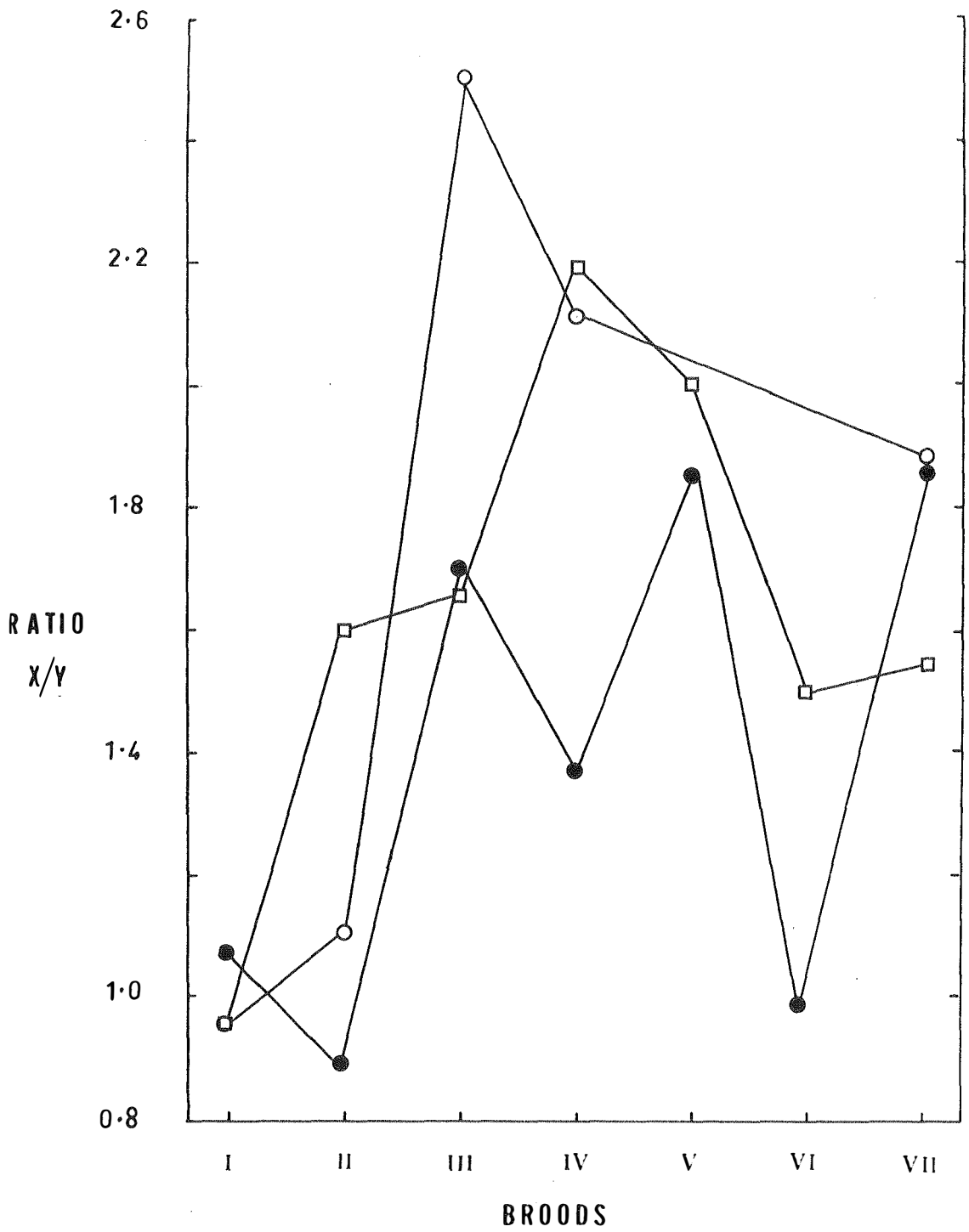


Figure 7. Change in ratios of X/Y in 2-day broods after transfer from 18°C to 25°C for three males no. 6, 9, 10.

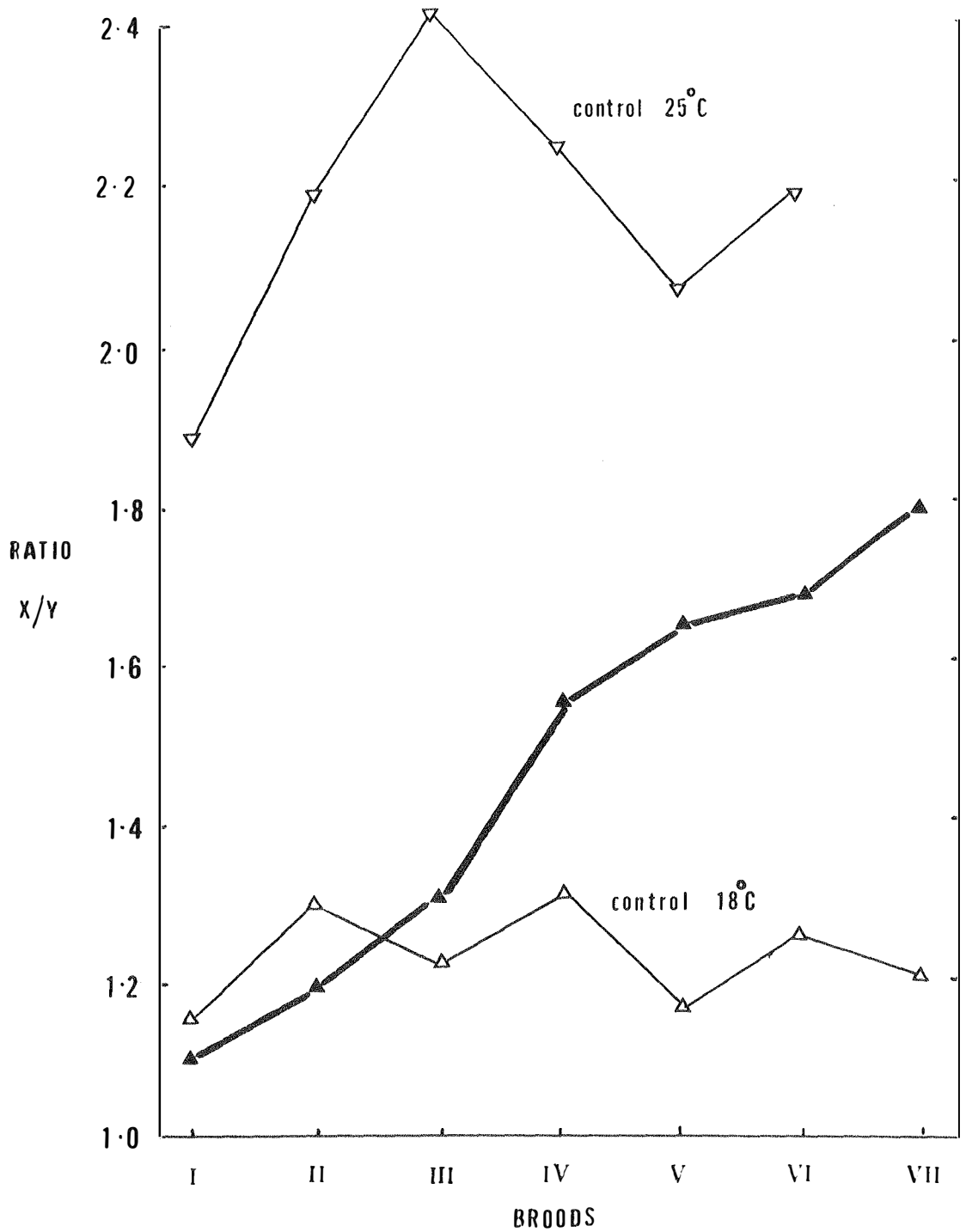


Figure 8. Change in mean ratios of X/Y in 2-day broods after transfer from 18°C to 25°C.

IV. DISCUSSION

Time of temperature sensitivity

The timing in the time of temperature sensitivity experiments is based on the autoradiographic study of spermatogenesis by Chandley and Bateman (1962). At the lower temperature of 18°C, spermatogenesis takes place at a rate of about one half to that at 25°C and the brood period at 18°C was thus four days instead of two as at 25°C. The relationship between spermatogenesis (for early and late utilization of sperms) and time is shown in Figure 9.

From Figure 10 in the temperature increase experiment, Brood IV is the first in which sperms utilized responded to temperature change. According to the early utilization scale in Figure 9, the corresponding stage in spermatogenesis is late primary spermatocytes. However, some cells did show some response to temperature as late as in spermatid stage in Brood III. From the reverse experiment, Brood XII shows response by cells at an advanced stage of spermatids. However, the figures available for this reverse experiment were not large enough to be quite reliable. In both experiments, the ratio of X/Y did not reach the values at the control temperatures. In the increase temperatures experiment, even in Brood VII, X/Y is only equal to 1.8, well outside the mean of 2.15 at 25°C. In the decrease temperature experiment, the lowest value of X/Y reached is 1.32 in Brood V as compared to the mean of 1.2 at the 18°C control temperature. In Broods VI and VII, X/Y did not differ from that in Brood V.

sperm ejac testis			spermatids			meiosis	spermatocytes (primary)			
10	9	8	7	6	5		4	3	2	1

Early utilization

Day	sperm ejac testis					spermatids			meiosis	spermatocytes (primary)			
	12	11	10	9	8	7	6	5		4	3	2	1

Late
utilization

I II III IV V VI VII BROODS

Figure 9. Time of spermatogenesis in D.melanogaster (after Chandley and Bateman).

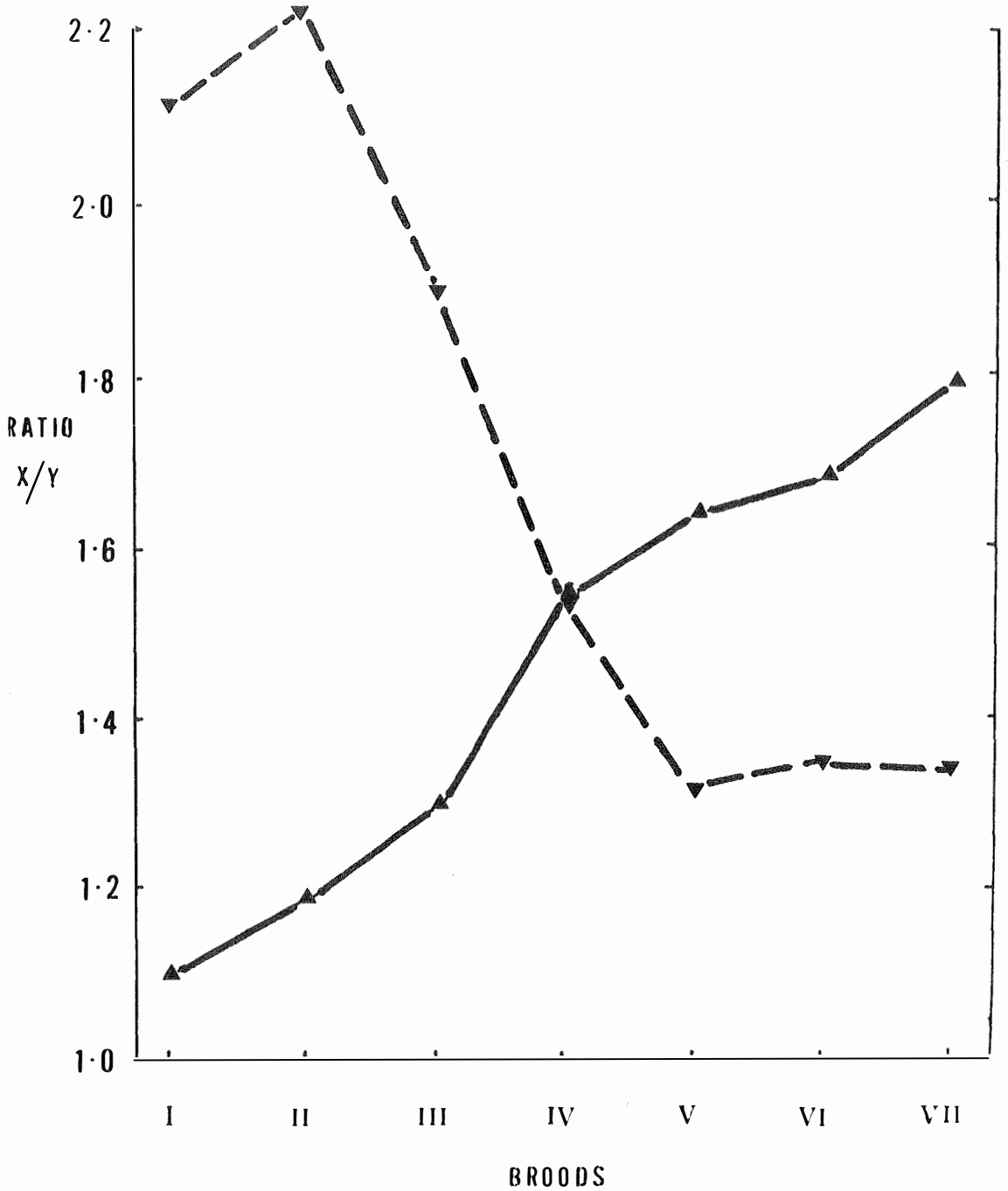


Figure 10. Time of response to temperature for Nonrandom Segregation. Broken line represents decrease temperature experiment and continuous line represents the reverse experiment.

In the above discussion, no mention is made regarding the ratio of O/XY since the figures obtained varied greatly even at constant temperatures.

The time of temperature sensitivity experiments, thus show that there is somewhat partial sensitivity to temperature change. This could be partly due to sperm retention by males. The result of these experiments is not quite accurate since the X/Y ratio itself varied at constant temperatures. Nevertheless, it does show quite conclusively that the time of sensitivity to temperature change lies within spermatogenesis between the stages containing spermatocytes and spermatids. However, the exact nature of the physical or chemical basis of this temperature affect is not known.

Sperm morphology

Cytological study shows that there is no difference between the morphology of sperms of males raised at 18°C and 25°C. Sperms were morphologically normal at both temperatures. This observation parallels that reported by Anderson (1967) who made an electron microscopic study of spermiogenesis in D. melanogaster cultured at 18°C and 26°C. He reported that there was no apparent morphological basis for sterility observed at elevated temperatures.

This observation supports the hypothesis that the effect of temperature on nonrandom segregation is merely to bring about random assortment of complementary gametes (X and Y, O and XY) into functional and nonfunctional poles and this change is therefore not due to any form of structural abnormality.

VIII. RESUME

1. The expression of Segregation Distortion is not affected by either decreasing or increasing of temperature.
2. The slight but consistent deviant sex-ratio associated with S.D. is also not sensitive to temperature changes.
3. Nonrandom Segregation is temperature sensitive. Lowering the temperature from 25°C to 18°C results in the production of equal frequencies of functional complementary gametes formed by sc^4-sc^8/Y males within disjunctional and nondisjunctional classes. Lowering the temperature also results in a decrease of the frequency of non-disjunctional gametes.
4. The effect of temperature on Nonrandom Segregation is reversible and in both cases, there is a lag of one generation.
5. The time of temperature sensitivity in Nonrandom Segregation is located between spermatocyte and spermatid stages in spermatogenesis.
6. No paternal age effect on Nonrandom Segregation is observed, at both lower and high temperatures.
7. No apparent morphological differences are observed between testes of sc^4-sc^8/Y males raised at 18°C and those raised at 25°C.

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SECTION TWO

I. ESTIMATION OF FREQUENCIES OF SUPERNUMERARY (B)

CHROMOSOME CARRIERS

a. INTRODUCTION

The presence of a mitotically stable supernumerary (B) chromosome in the local populations of the short-horned grasshopper Phaulacridium vitatum was first detected as early as in 1956 in the Hobart Botanical Gardens. The frequency of carriers of these B chromosomes in this population ^{was} were estimated for 9 successive years by Jackson (in Cheung 1966). In 1965, three new populations were studied and the frequency of their B carriers estimated (Cheung 1966). In all instances above, the estimation of the frequency of B carriers was made through the study of meiosis in the male grasshopper. The aim of this part of the work is to estimate the frequency of B carriers in the female population.

The precocity and positive heteroplicosity of the B and X chromosomes relative to the autosomes in meiotic divisions allow carriers to be easily recognised in meiotic prophase. Hence an accurate estimation of the frequency of carriers of the B chromosome in the males can be easily made. In the female, the study of meiotic stages is technically difficult although this has been done (White, Cheney and Key 1963). Even though preparations of meiotic chromosome can be made from females, only a few cells at the required stage can be obtained from one female. Hence the results obtained are likely to be inaccurate. In any case the labour involved is very great.

I have therefore decided to estimate the frequency of carriers in the female from a study of mitotic divisions of the primary germ cells and oögonia in the germarium of the ovary. Two to three ovarioles give about two to three dozen cells in mitotic metaphase from which chromosome counts can be made more reliably and with less labour than by a study of meiosis. It is interesting to note that it is not necessary to make a total chromosome count of a cell; the B chromosome is very large relative to the rest of the complement and its presence can be reliably detected (Figure 2).

b. MATERIALS AND METHOD

100 females were sampled from the Botanical Gardens population within a period of two weeks. 0.2 mls. of 0.04% colchicine in Ringer solution A were injected into the abdomen of the females using a micrometer syringe. The females were then left for five hours, at the end of which the abdomen was dissected, the ovaries removed and immediately subjected to hypotonic treatment by placing them in distilled water for five minutes. The ovaries were then fixed in Carnoy (1886) consisting of (v/v) 100 absolute alcohol, 16 glacial acetic acid and 50 pure chloroform. The fixed ovaries could be stored at 0°C in the fixative for a period of at least ten months.

Squash preparations of the germarium (Figure 1) containing the primary germ cells and oögonia were made in orcein. An average of 10 cells were scored from each female grasshopper.

96 male grasshoppers were also sampled from a different population,

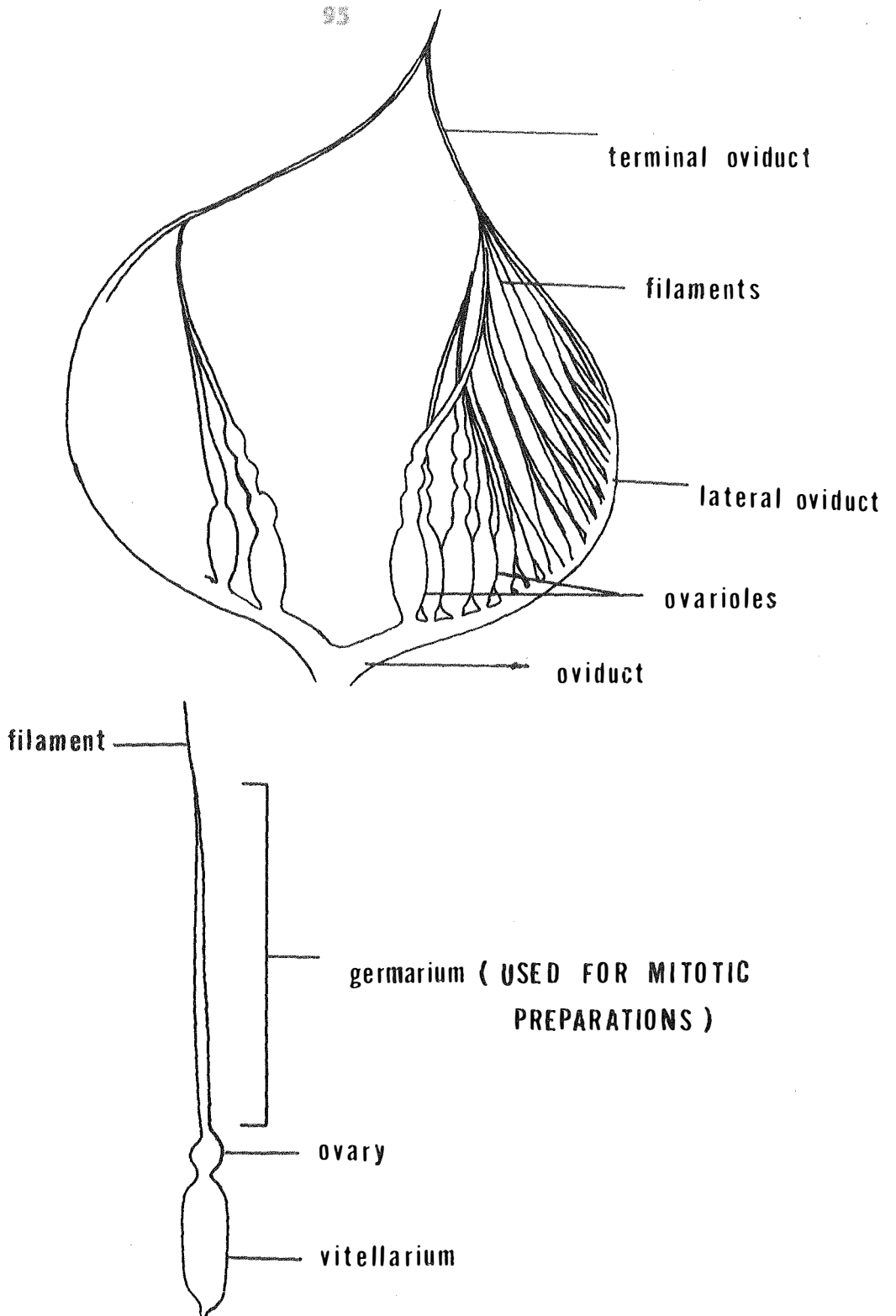


Figure 1. Diagram showing reproductive organs of a female grasshopper, P. vitattum.

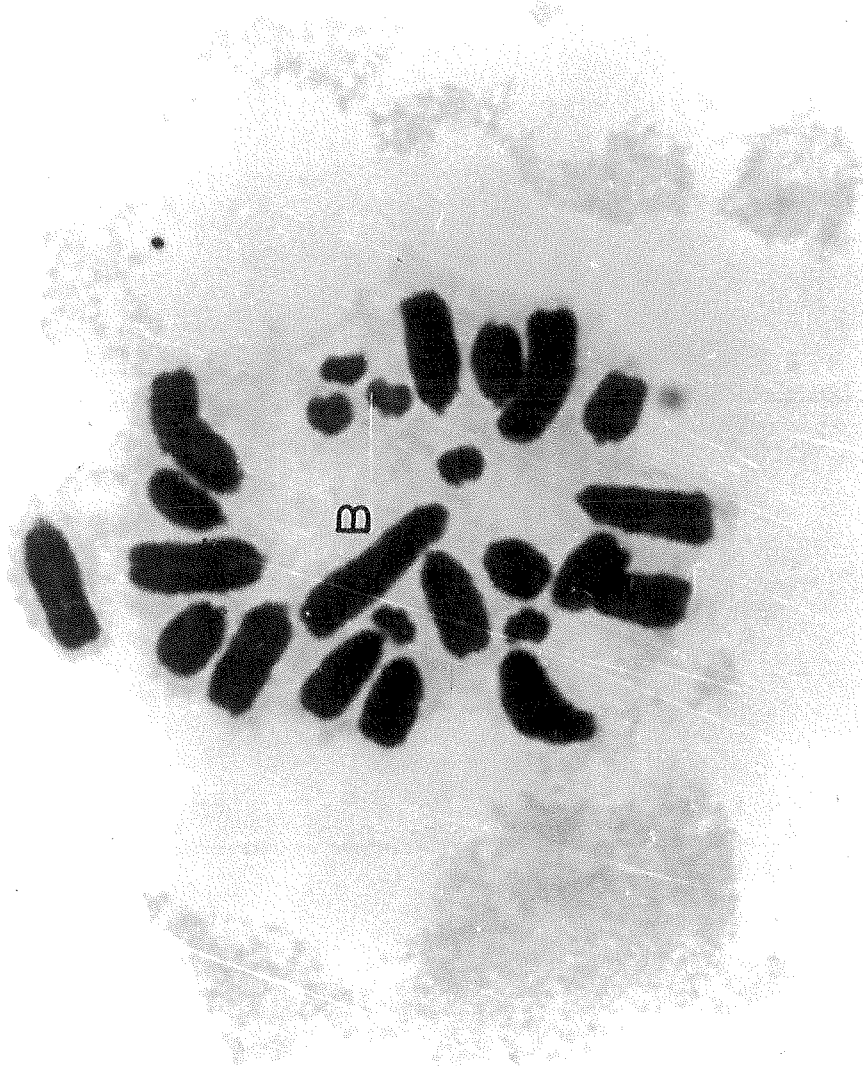


Figure 2. P. vitattum . Mitotic metaphase showing supernumerary (B) chromosome in the centre.

Woolden Place. Testes were removed and placed in distilled water for hypotonic treatment. They were then fixed in Carnoy (1886) as above. Squash preparations were made in orcein.

c. RESULTS

Data for the frequency of +B females in the female population in the Botanical Gardens and also of +B males in the male population in Woolden Place are given in Table 1.

Table 1. Frequencies of +B individuals in two populations of P. vitattum, sampled in early 1967.

Population	sex	-B individuals	+B individuals	total	frequency of +B individuals
Botanical Gardens	♀	94	6	100	0.060
Woolden Place	♂	87	9	96	0.094

Karyotype of P. vitattum female

In P. vitattum, the normal female complement is $2n = 22 + 2X$ (Sharman, 1952). At mitotic metaphase, the two X's are not distinguishable from the autosomes. The morphology of the autosomes is similar to that of the male autosomes, three pairs are extremely small, reduced to little blobs in the colchicine treated cells. Four pairs are of medium size while the remaining four are fairly large (Figure 2). All members of the complement are acrocentric.

d. DISCUSSION

Cheung (1966) calculated a theoretical value for the frequency of B carriers in the female population of P. vitattum. This calculation was based on the 7:3 ratio of the movement of X and B chromosomes at Anaphase I. He found that the frequency of a single B carrier among the females would be 0.0865 in the 1965 meiotic season and this figure would decrease at the rate of 0.0235 per generation. Based on this calculation (this is logical since the 7:3 ratio of the movement of X and B chromosomes during Anaphase I persisted in the 1967 meiotic season, see page 100), the frequency of +B individuals among females in the Botanical Gardens population in the 1967 meiotic season would be \approx 0.0395. (It is assumed that there is only one generation per annum).

The frequency of +B females is found to be 0.060 showing a deviation from the expected figure by 0.0205. The B chromosome therefore, seems to be maintained in the population at a higher frequency than expected. This, as Cheung had realized in the case of the males, suggests that the presence of the single B chromosome in association with certain background genotypes and also certain environmental conditions renders the +B females their fitness, which enables them to be significantly maintained in the population.

The frequency of the +B males in the Woollen Place population in the 1967 meiotic season is 0.094 as compared to 0.1650 in 1965. The frequency should increase at the rate of 0.015 per generation

(Cheung, 1966). The fact this did not take place suggests that there might be a strong selection against the +B male carriers, or alternatively, B chromosomes appear to effect a reduction in fitness of those males which possess them. However, a much larger sample is required for study to confirm this.

II. DISTORTIONAL SEGREGATION OF B CHROMOSOMES

In spermatogenesis, definite associations between B and X chromosomes during late Prophase I are observed. Cheung (1965) found that there were three categories of B and X associations. The two chromosomes could be found either together (end-to-end or side-by-side) or separate from each other. It was observed that the frequency of the two chromosomes found in an end-to-end and side-by-side associations was ≈ 0.7 . The B and X chromosomes are also observed to have a definite Anaphase I movement. Cheung found that the frequency of them going to the opposite pole was ≈ 0.7 and that to the same pole ≈ 0.3 .

An attempt was made to test the stability of these associations between B and X chromosomes at late Prophase I and also at Anaphase I. The results are shown in Tables 2-4.

χ^2 tests show that both associations do not differ significantly in the two meiotic seasons. It seems therefore that the transmission of B chromosomes from one generation to the next is fairly constant, and hence further variation in +B frequencies in *P. vittatus* populations could be due to other factors, such as selection.

From the frequencies of the associations of B and X chromosomes during Prophase I and during late Anaphase I, it appears that there is a direct relationship between these two associations. It can be confidently inferred that when the B and the X chromosomes do synapse during Prophase I (either end-end or side-side), they usually disjunct

during Anaphase I and consequently segregate into opposite poles; the frequency of synapsis to the frequency of disjunction is $\approx 0.7:0.7$. This situation in which synapsis usually leads to disjunction is comparable to that found in Nonrandom Segregation shown by $\ln(1)sc^4-sc^8/Y$ males in D. melanogaster (see page 4).

Table 2. Association between B and X chromosomes during late Prophase I.

Type of Association	Number of cells	Frequency
Separate	75	0.2885
End-End	30	0.1153
Side-Side	155	0.5962
Total	260	1.0000

Table 3. χ^2 for agreement of frequencies of B and X association with 7:3 ratio.

	Separate	End-End or Side-Side	Total
obs.	75	185	260
Exp.	78	182	260

$$\chi^2_{(1)} = 0.1649 \quad df = 1$$

$$P > 0.5$$

N.S.

Table 4. Frequencies of movement of B chromosomes with respect to that of X chromosomes at late Anaphase I.

	Same Pole	Opposite Pole	Frequency	Total
Obs.	91	181	0.3346	272
Exp.	81.6	190.4	0.6634	272

$$\chi^2_{[1]} = 1.547$$

$$df = 1$$

$$P > 0.2$$

N.S.

III. CYTOGENETIC EFFECTS OF B CHROMOSOMES IN

PHYLACRIDZUM VITATUM - INFLUENCE ON CHIASMA

FORMATION

a. INTRODUCTION

Since the time they were first discovered by Wilson in 1905 in the hemipteran insect Metapodius, accessory chromosomes (as they were originally called) had been under intensive study. They differ from the normal chromosome complements not only in that they form a system of mutation and recombination of their own, but also in that they defy the Laws of Mendelian Inheritance and selection. Although much have been discovered about their behaviour, origin, distribution and significance in population dynamics, the same can hardly be said about their effects, either cytological or genetic, on the individuals which possess them.

Muntzing (1959) found that B chromosomes reduced the fertility and vigour in rye, and this effect increased with the number of B chromosomes present. Similar results were reported by Kayano (1956, 1957) and Kimura and Kayano (1961). On the other hand, B chromosomes were found to be advantageous in certain plants. This is illustrated in the case of Festuca pratensis (Bosemark 1957a and 1957b), Centaurea scabiosa (Frost 1958), Trillium spp (Rutishauser in Muntzing 1959) and Achillea spp (Muntzing, 1959) in which the fertility of the carrier plants was enhanced. B chromosomes have also been known to affect

development. Thus in Polyceelis tenuis, a freshwater turbellaria (Melander, 1950), the rate of development and sexual maturity were decreased causing reproductive isolation. However, in the mealy bug Pseudococcus obscurus, developmental time was found to increase in the males with a single B chromosome (Nur 1966a and 1966b). At the same time, there was a reduction in the number of sperms produced. No such effects of B chromosomes were observed in the females.

Many of the genetic effects of B chromosomes have been found to occur at the endophenotypic level as suggested by Lewis and John (1959). Thus in Pyrogorphia dispar, it was found that A chromosomes were more subject to numerical error in -B individuals. On the other hand, a direct effect of B chromosomes on the stability of A chromosomes has been observed in Achillea (Ehrenborfer in John and Hewitt 1965) and also in Trillium and Crepsis (Rutishauser in John and Hewitt 1965). Jackson and Newmark (1960) found that one of the two types of B chromosomes found in Haplopappus gracilis brought about the production of pigments in the achene wall.

The influence of B chromosomes on the level of chiasma formation was first investigated by Baker (1960) who found that in the mottled-grasshopper Myrmeleotettix maculatus, +B individuals have significantly higher chiasma frequency than -B individuals. In this part of the thesis, an attempt is made to compare the levels of chiasma formations among -B and +B individuals in the short-horned grasshopper, P. vitattum.

b. MATERIALS AND METHOD

Of the four populations sampled in the 1965 meiotic season, the chiasma frequencies of only two of them viz. Botanical Gardens and Woolden Place populations were satisfactorily estimated. Slides of the other two populations were available but too few for the purpose of this work. The chiasma frequency of one population in the 1967 meiotic season i.e. Woolden Place population, was also estimated.

In the male P. vitattum, the autosomes are made up of three small and eight fairly large chromosomes. Preliminary observations revealed that each of the three small autosomes did not have more than one chiasma. Of the eight large autosomes, only four seemed to show, quite consistently, two or more chiasma per autosome. Each of the remaining four, usually had a single chiasma. Chiasma formations were not easily observed in the B and X chromosomes. This was due to their precocious meiotic behaviour. In view of all these observations, it was decided to make chiasma counts of only the four largest autosomes. Ten diplotene cells per individual male were scored. The data is given in Table 5. Only males with one B chromosome were scored.

c. RESULTS

From the results, shown in Tables 6 - 10, the following conclusions can be made:

Table 5. Chiasma frequencies of the four largest chromosomes of *P.vitattum* in three populations. \bar{X}_a = the average value of ten cells per individual. Log V = the log of the variance of chiasma frequency within an individual.

Population	Botanical Gdn. 1965		Woolden Place 1965		Woolden Place 1967	
Frequency of B carriers	10.53%		16.5%		9.37%	
	\bar{X}_a	log V	\bar{X}_a	log V	\bar{X}_a	log V
-B	4.4	-0.5735	4.0	-0.3279	4.3	-0.6326
	4.5	-0.5560	4.5	-0.5229	4.8	-0.2061
	4.5	-0.3251	4.6	-0.5229	4.8	-0.7496
	4.6	-0.1972	4.8	-0.7799	4.9	-0.4921
	5.4	-0.5735	4.9	-0.1152	5.0	-0.3526
			5.0	-0.6578	5.1	-0.2644
	5.5	-0.5638	5.0	-0.3119	5.2	-0.3979
	5.5	-0.2434			5.2	-0.3979
	5.5	-0.5560	5.2	-0.7496	5.2	-0.3979
	5.8	+0.2270	5.3	-0.6144	5.4	-0.5735
+B	5.8	-0.1046	5.4	-0.5735		
	6.1	-0.4921	5.6	+0.0233	4.9	-0.2644
	6.2	-0.7496	5.6	-0.5735	5.1	-0.1152
			5.7	-0.6234	5.5	-0.5560
			5.8	-0.3979	5.5	-0.0250
			6.8	-0.3979	5.6	-0.3107
					5.8	-0.7979
					6.2	-0.2061
					6.2	-0.2061
Mean chiasma frequency	-B 4.68		4.67		4.99	
	+B 5.77		5.68		5.60	
Population mean \bar{X}_a freq.	5.32		5.21		5.26	
Variance between individual	-B 0.1670 0.2680		0.1283 0.2959		0.0996 0.2472	
	+B 0.0857 0.1982		0.2479 0.3247		0.2171 0.1713	
Population variance between individual	0.1182 0.2261		0.1927 0.3114		0.1493 0.2140	
Mean log V	-B -0.4451		-0.4626		-0.4465	
	+B -0.2118		-0.4885		-0.3102	
Population mean log V	-0.3089		-0.4764		-0.3859	

1. In all the three populations, the mean chiasma frequencies among the +B individuals are significantly different from the mean chiasma frequencies among the -B individuals. In all cases, +B individuals have a higher mean chiasma frequency (Table 6).
2. The mean chiasma frequencies among the +B individuals are not significantly different in the two populations in the 1965 meiotic season (Table 7).
3. The mean chiasma frequencies among the -B individuals are also not significantly different in the two populations in the 1965 season. Here, a test of significance is unnecessary since the two means are 4.68 and 4.67 with a very small deviation of 0.01.
4. In the Woolden Place population, the mean chiasma frequencies among -B and +B individuals in the 1965 season do not differ significantly from the corresponding values in the 1967 season. The chiasma frequencies among -B and +B individuals were therefore relatively constant at least for the two meiotic seasons, 1965/1967 (Table 8).
5. The population mean chiasma frequencies of the two populations in the 1965 season do not differ significantly (Table 9).
6. The population mean chiasma frequencies of Woolden Place population do not differ in the years 1965 and 1967 (Table 10).

Table 6. A comparison of mean chiasma frequencies between -B and +B individuals in three populations in 1965 and 1967.

Population	Mean chiasma freq.		t	df	P
	-B	+B			
Bot. Gardens (65)	4.68	5.77	5.42	10	≤ 0.01 ***
Woolden Place (65)	4.67	5.68	4.45	13	≤ 0.01 ***
Woolden Place (67)	4.99	5.60	3.33	16	< 0.01 ***

Table 7. A comparison of mean chiasma frequencies between +B individuals in two populations (Bot. Gardens and Woolden Place) in 1965.

Mean chiasma freq.		t	df	P
B.G.	W.P.			
5.77	5.68	0.418	13	> 0.5 N.S.

Table 8. A comparison of mean chiasma frequencies between (i) -B individuals and (ii) +B individuals in Woolden Place population in two years 1965/67

	Mean chiasma freq.		t	df	P
	1965	1967			
-B	4.67	4.99	1.964	15	> 0.05 N.S.
+B	5.68	5.60	0.332	14	> 0.7 N.S.

Table 9. A comparison of population mean chiasma frequencies and population variance, between two populations in 1965

	B.G.	W.P.	t	V.R.	df.	P
Chiasma freq.	5.32	5.21	0.710		23	> 0.3 N.S.
Variance	0.1182	0.1927		1.63	13/10	> 0.2 N.S.

Table 10. A comparison of population mean chiasma frequencies between 1965 and 1967 Woolden Place populations.

Pop. mean chiasma freq.		t	df	P
1965	1967			
5.21	5.26	0.348	29	> 0.7 N.S.

From the above analysis, it is reasonable to conclude that B chromosomes seem to raise the level of mean chiasma formation of individuals possessing them. This influence of the B chromosomes is found to exist to the same degree in the two geographically isolated populations studied and is constant from one generation to the next.

A comparison of variances between individuals, of -B and +B individuals leads to the following conclusions:

7. There is no significant difference found between variance of -B and +B individuals in all the three populations (Table 11).

8. There is also no significant difference between (a) variance of -B individuals (b) variance of +B individuals in the two populations in the 1965 meiotic season (Table 12).

9. The population variance between the two populations in the 1965 meiotic season do not differ significantly (Table 9).

It appears therefore, that although the B chromosomes raised the level of chiasma formation, they did not have any influence on the variance between individuals with respect to chiasma frequencies.

Table 11. A comparison of between-individual variation between -B and +B individuals.

Population	Variance		V.R.	df.	P
	-B	+B			
Bot. Gardens (65)	0.1670	0.0857	1.95	4/6	> 0.2 N.S.
Woolden Pl. (65)	0.1283	0.2479	1.93	7/6	> 0.2 N.S.
Woolden Pl. (67)	0.0996	0.2171	2.18	7/9	> 0.1 N.S.

Table 12. A comparison of between-individual variation between (1) -B and (11) +B individuals in two populations in 1965 meiotic season

	Variance		V.R.	df	P
	B.G.	W.P.			
+B	0.1670	0.1283	1.30	4/6	> 0.2 N.S.
+B	0.0857	0.2479	2.89	7/6	> 0.1 N.S.

The log variance (Log V) of chiasma frequencies is calculated for each -B and +B individual. This log v is actually the degree of within-individual variation i.e. the degree of stability of chiasma formation within an individual. From Tables 13-17, the following conclusions are noted:

10. In all the three populations studied, the mean log v among -B individuals do not differ significantly from the corresponding values among +B individuals (Table 13).

11. The mean log v among -B individuals do not differ significantly between the two populations in the 1965 meiotic season. Similarly, the mean log v among +B individuals are not significantly different between the two populations (Table 14).

12. In the Woolden Place population, the mean log v among the -B individuals did not change from the 1965 to the 1967 seasons. The same is also true for +B individuals (Table 15).

13. The population mean log v among all three populations do not differ significantly (Tables 16 and 17).

Table 13. A comparison of mean log v between -B and +B individuals in three populations in 1965 and 1967.

Population	Mean log v		df	t	P
	-B	+B			
Bot. Gardens 65	-0.4451	-0.2118	10	0.838	> 0.3 N.S.
Woolden Pl. 65	-0.4626	-0.4885	13	0.0897	> 0.9 N.S.
Woolden Pl. 67	-0.4465	-0.3102	16	0.621	> 0.5 N.S.

Table 14. A comparison of mean log v between (i) -B individuals,
(ii) +B individuals in two populations in 1965

	Mean log v		df	t	P
	B.G.	W.P.			
-B	-0.4451	-0.4626	10	0.056	> 0.9 N.S.
+B	-0.2118	-0.4885	13	1.036	> 0.3 N.S.

Table 15. A comparison of mean log v between (i) -B individuals
(ii) +B individuals in Woolden Place population in two
years 1965/67

	Mean log v		df	t	P
	1965	1967			
-B	-0.4626	-0.4465	15	0.041	> 0.9 N.S.
+B	-0.4885	-0.3102	14	0.716	> 0.3 N.S.

Table 16. A comparison of population mean log v between two populations
in 1965.

Population mean log v	df	t	P
B.G.	W.P.		
-0.3089	-0.4764	25	0.8615 > 0.3 N.S.

Table 17. A comparison of population mean log v between 1965 and 1967 Woolden Place populations.

Population mean log v		df	t	P
1965	1967			
-0.4764	-0.3859	31	0.527	> 0.5 N.S.

B chromosomes do not seem to influence the within-individual variation with respect to chiasma formation in the P. vitattum male. (However, in two populations i.e. Bot. Gardens 1965 and Woolden Pl. 1967, +B individuals have a slightly higher mean log v although these differences are not really significant. It is only in the Woolden Pl. 1965 population that the mean log v among +B individuals is lower than that of among -B individuals. Even then, this difference is extremely small).

d. DISCUSSION

The effect of B chromosomes on the level of chiasma formation is shown in Figure 3. It can be seen that the +B individuals have a much higher mean chiasma frequency than the -B individuals. It is also clear that the between individual variation is somewhat equal in the two populations (+B and -B).

Baker (1960) working on the grasshopper Myrmeleotettix maculatus reported that B chromosomes raised the chiasma frequencies of

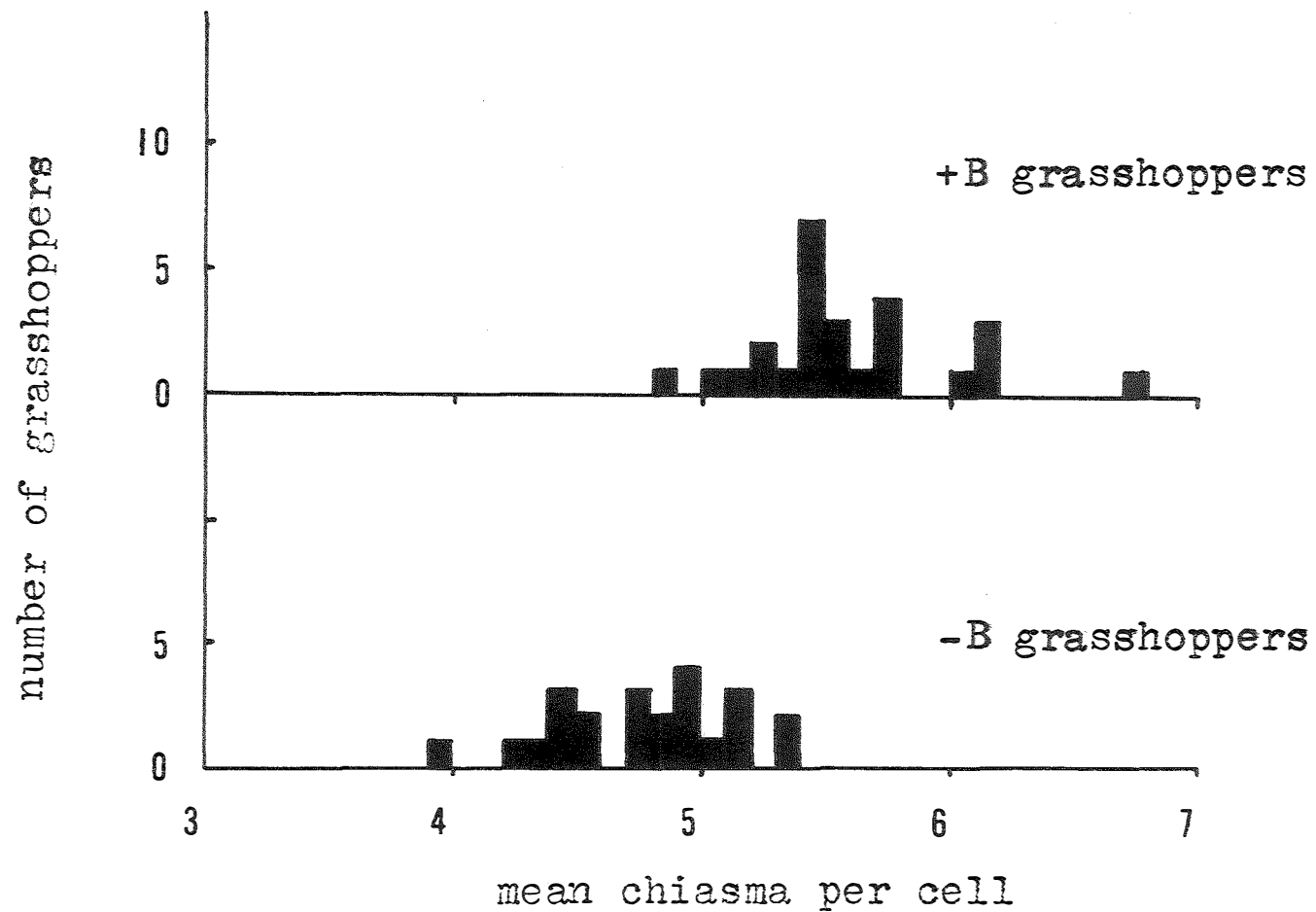


Figure 3. Histograms showing chiasma frequency in 22 -B and 23 +B grasshoppers.

individuals which possessed them. John and Hewitt (1965) who revised the work of Baker, pointed out that this was not necessarily true. They found that B chromosomes merely raised the mean chiasma frequency of +B individuals and that some +B individuals did have lower chiasma frequencies than a number of -B individuals. Studies of the B chromosome system in P. vitattum support the view presented by John and Hewitt (Table 5). The range of chiasma frequencies in +B individuals is 4.9 - 6.8 and in -B individuals is 4.0 - 5.4. As in M. maculatus, the B chromosomes of P. vitattum seem to cause the breakdown of the control mechanism of chiasma formation resulting in an increase in the mean level of chiasma formation in individuals possessing them.

John and Hewitt (1965) also found that the populations studied differed significantly in mean chiasma frequency and that if +B individuals were omitted from the analysis, the differences were still evident. Similar situations are not found in P. vitattum. The three populations studied show no significant differences in population mean chiasma frequencies and this situation persists when +B individuals are omitted from the analysis.

Between-individual variations with respect to chiasma formation is not significantly affected by the presence of B chromosomes. This suggests the stability of the influence of the B chromosome on the level of chiasma formation. This result is in contrast to that of John and Hewitt (1965) who reported an increase in the between-individual

variation associated with B chromosomes in M. maculatus.

In P. vitattum, B chromosomes do not seem to have any effect on the within-individual variation with respect to chiasma frequency. Nevertheless, the results (Table 5) do show a trend of increase in this within-individual variation among +B individuals although this increase is not significant. John and Hewitt (1965) reported that the within-individual variation in chiasma frequency showed an increase in the presence of B chromosomes.

The frequency of +B individuals in a population does not seem to have any influence on either the mean chiasma frequency or the within-individual variation with respect to chiasma frequency. However, more populations are required for study.

IV. RESUME

1. The frequency of +B individuals among females in the Botanical Gardens population in the 1967 meiotic season is found to be about 0.06.
2. Associations between B and X chromosomes during late Prophase I and also Distortional Segregation of B chromosomes with respect to X chromosomes at Anaphase I have been fairly constant, at least in the two meiotic seasons studied (1965/1967).
3. B chromosomes appear to raise the mean chiasma frequency of individuals possessing them. This effect is maintained in the following generations.

4. B chromosomes have no significant influence on the between-individual variation with respect to chiasma frequency.
5. The within-individual variation in chiasma frequency i.e. the stability of chiasma formation within an individual, is not influenced by the presence of B chromosomes.
6. The frequency of +B individuals in a population does not influence the mean chiasma frequency or its between-individual variation.

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